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**(54) BACTERIUM DETECTOR**

(57) An object of the present invention is to offer a microorganism-detecting apparatus where the microorganism causing a food poisoning can be detected/identified by a simple operation and also a disposal treatment can be conducted safely and surely. Means for achieving the object is as follows: Thus, the apparatus for detecting microorganism as mentioned below is portable and enables us to selectively incubate and detect the microorganism (particularly those which are a cause of food poisoning) without skillfulness and the detecting apparatus containing pathogenic microorganism (such as that which is a cause of food poisoning) can be safely and surely disposed. Said apparatus is characterized in having at least (a) a container (1) for holding a medium which is used for culturing the microorganism to be detected during the period of the incubation of said microorganism; (b) a microorganism-collecting part (6); (c) a means (3) for making possible a process of receiving, in a noncontact manner with the microorganism-collecting part, the medium used for culturing the microorganism to be detected until the incubating stage of the microorganism; and (d) a means (9) for disinfecting the medium after incubation.

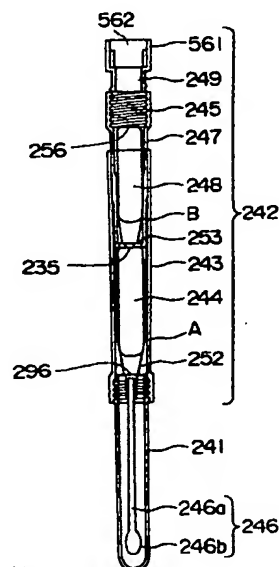


FIG. 18

## Description

TECHNICAL FIELD

5 The present invention relates to an apparatus for selectively detecting microorganisms whereby pathogenic microorganisms, particularly food poisoning microorganism and drug-resistant microorganism such as methicillin-resistant Staphylococcus aureus (MRSA) can be easily and simply detected and/or identified. More particularly it relates to an apparatus for a selective detection of microorganisms which is particularly suitable even for private and domestic use where its storage for long time can be expected, which can be immediately used at any time when needed, which has  
10 a portable form which can be conveniently and easily carried, which is possible for safely detecting and identifying a food poisoning microorganism or the like by a simple operation and which can be disposed safely, surely and easily after use.

BACKGROUND ART

15 Pathogenic microorganisms which are present in living environment have been considerably overcome due to a high development in medical service and progress in therapy in recent years particularly inventions and developments in antibacterials and antibiotics and, moreover, due to an improvement in hygiene. However, once they break out, big damage and fatal problem may result and they are still causes of big problems.

20 Examples of the pathogenic microorganisms which cause troubles when present around us are those causing food poisoning and those resistant to antibiotics such as methicillin-resistant Staphylococcus aureus (MRSA) and the like. Thus, there are problems such as occurrence of a lot of patients infected by them and hospital infection.

Taking food and beverage or the "eating" action is essential for animals including human being and, therefore, in spite of progress in science and high development in technique in recent years, it is still difficult to completely prevent  
25 "food poisoning" caused by taking food and beverage. On the other hand, prevention of food poisoning as such has been more and more important because of the Product Liability Law which has been inaugurated recently.

Food poisoning is roughly classified into three depending upon its cause, i.e. that by natural poison (toxin) (such as swellfish poison (Tetraodontidae toxin) and mushroom poison), that by putrefaction of food and beverage (such as by decomposed products of food and beverage) and that by contamination by or proliferation of bacteria (such as Staphy-  
30 lococcus, Salmonella and Vibrio parahaemolyticus) in food and beverage. Among them, food poisoning by natural poison can be easily avoided by preventing the intake of food/beverage containing the causing poison. In addition, detection of putrefaction of food/beverage by means of checking the changes in appearance, smell, etc. is usually easy. Therefore, prevention of food poisoning due to putrefaction of food/beverage is relatively easy in many cases.

In contrast to those two types of food poisoning, contamination by or proliferation of the microorganism is usually  
35 invisible in the case of food poisoning by bacteria (that is, the bacterial food poisoning) and, moreover, detection of said contamination or proliferation is very difficult without participation of professional people. Accordingly, said bacterial food poisoning is a food poisoning which is most difficult to avoid.

Occurrence of food poisoning in facilities such as restaurants, caterers for luncheon and feeding facilities affects on significantly many people and, in addition, the symptom is serious such as severe vomiting, diarrhea, abdominal pain,  
40 high fever, etc. whereby it is apt to result in a particularly serious problem such as stop of business of said facility for long term. Further problem is that, since the microorganism causing such food poisoning produces toxic substances such as enterotoxin in the body of infected patients, it is necessary to conduct a therapy after infection and, moreover, said therapy is very often accompanied by a difficulty.

Accordingly, it is quite necessary that the microorganism which is a cause of such a symptom is found at an early  
45 stage and that a due preventing measure such as disinfection is conducted for preventing the poisoning.

As a result of the use of antibacterials and antibiotics, occurrence of diseases, especially infectious diseases such as contagious disease, has significantly decreased. On the other hand, however, many resistant microorganisms have appeared as a secondary phenomenon resulting in a big problem. There is a problem that, when a powerful antibiotic substance is developed, resistance thereto occurs immediately and, in addition, hospital infection by such resistant  
50 microorganisms has become a big problem too. Since patients including those suffering from severe disease and aged people has a decreased resistance, microorganisms which are resistant to antibiotics cause a intractable infectious disease to them whereby, even when the infection is found, there will be no effective therapy and that is a big problem. For preventing the hospital infection caused by such resistant microorganisms, it is necessary to specify the causing microorganism in early stage of infection, to find its presence at an early date and to effectively and appropriately prevent the  
55 infection by means of disinfection, etc. Accordingly, it is necessary for hospitals to periodically check whether such microorganisms appear in the medical environment.

Up to now, there has been no means other than common preventing measures (such as keeping the cooking utensils, e.g. kitchen knife and chopping board, clean and also keeping hand and fingers of the persons participating in

cooking clean) for preventing the food poisoning. Even at present, when food poisoning occurs, only an *ex post facto* measure (i.e. the causing microorganism is detected and confirmed by the search (by incubation of the suspected microorganism found in the food/beverage) of the public health center controlling the place where the poisoning occurred) is available. In addition, detection and confirmation of the causing microorganism by the center as such usually require quite a long period (e.g., around one to two weeks). Further, the search by the public health center needs a "prescribed procedure" and, moreover, in view of honor or reputation (such as an entire loss of customers by "rumor"), such a procedure is not always fully activated. In another viewpoint of usual countermeasure, it is not always possible to detect and confirm so simply.

In the investigation at the professional organizations such as public health centers and hospitals, a method which has been adopted is that various samples as mentioned above and those collected from patients and from medical environment are incubated on agar plates using a selective medium and the colonies formed on the medium are observed by naked eye or that the microorganism incubated as such is confirmed by other detecting and confirming means. Recently, a judging method using polymerase chain reaction (PCR) has been adopted as well. However, the operations are troublesome in all of such conventional methods and, in judging the colonies grown on the agar medium, professional technique and skillfulness are needed for identification. In addition, professional technique and skillfulness as well as specific devices or the like are required for the operations from collection of the sample to its incubation and there is a disadvantage that total cost for the detection is high. Further, in the case of a method utilizing a PCR, it is again necessary to apply professional technique and skillfulness and there is another problem that, for certain microorganisms, discrimination of them from nonpathogenic ones is very difficult.

For a device for solving these problems, an apparatus accommodating a liquid medium enclosed in a capsule and a tampon for collecting microorganisms in a capsule-container is proposed, for example, in Unexamined Japanese Patent Publication Sho 62-171671 (JP Sho 62-171671 A). Because such an apparatus contains pathogenic microorganisms after incubation, however, it is difficult to discard it. Therefore, in the prior art the used apparatus has been discarded after disinfecting steps, including opening the used container followed by addition of a disinfectant solution to the medium containing the microorganisms.

Although the apparatus has been brought into an open system, for example, by taking off a stopper, etc., in order to discard the incubation system containing the pathogenic microorganisms, such a treatment is not only troublesome and risky but also requires professional technique and great skill, even installations. Furthermore, since, in addition to the container for incubation, it is always necessary to prepare a disinfectant solution for the treatment, it is troublesome in view of portability and convenience in use.

#### SUMMARY OF THE INVENTION

An object of the present invention is to provide an apparatus (or device) for detecting microorganisms whereby pathogenic microorganisms such as those causing food poisoning and resistant microorganisms can be detected and identified by simple operations and, after use, said apparatus can be safely and conveniently disposed.

Another object of the present invention is to provide an apparatus for detecting microorganisms which can be not only used in hospitals but also used privately or at home and which can be disposed by anyone easily and safely.

Still another object of the present invention is to provide an apparatus for detecting microorganisms where its handling is simple whereby it can be used at any time, previous or spontaneous countermeasure to pathogenic microorganisms can be made very easy and disposal of the apparatus after use can be made easily and safely.

The present inventors have conducted an intensive study and found that the following constitution is very effective in achieving the above-mentioned objects. Thus, the constitution is that the characteristic feature of a relatively convenient microorganism-detecting apparatus (wherein a medium which is to give an incubating condition suitable for the pathogenic microorganisms to be detected (such as specific microorganisms for food poisoning and microorganisms which are resistant to specific antibiotics) upon contacting and a microorganism-collecting part are located in a non-contact manner each other and, when incubation for detecting the microorganism is conducted, said specific medium and microorganism-collecting part are made contacted each other) is more fully utilized and, in addition, disposal (in some cases, disinfecting treatment) of the apparatus after use can be conducted safely and easily.

The apparatus for the detection of microorganisms in accordance with the present invention is based upon the above finding.

In one of its aspects, the present invention provides:

(1) an apparatus for detecting microorganism, characterized in that, said apparatus comprises at least:

- (a) a container for holding a medium which is used for culturing the microorganism to be detected during the period of the incubation of said microorganism,
- (b) a part where the microorganism is collected,

(c) a means for allowing us to holding the medium for culturing the microorganism to be detected, in a noncontact manner with the microorganism-collecting part (b), until the incubating stage of the microorganism, and  
(d) a means for disinfecting the medium after incubation;

(2) the apparatus for detecting microorganism according to the above (1) wherein the means (d) (means for disinfecting the medium after incubation) includes means for allowing a disinfectant to be held, without contact with the microorganism, until the completion of the incubation for the microorganism to be detected;

(3) the apparatus for detecting microorganism according to the above (1) or (2) wherein the medium and the disinfectant are made in such a manner that they can unitedly contact each other, by the action of a force applied from outside, after incubation of the microorganism;

(4) the apparatus for detecting microorganism according to any of the above (1) to (3) wherein the microorganism-collecting part (b) and the medium used for culturing the microorganism to be detected are made in such a manner that they can unitedly contact each other, by the action of a force applied from outside, during the incubation of the microorganism;

(5) the apparatus for detecting microorganism according to any of the above (1) to (4) wherein said apparatus contains:

(a) a hollow container having an opening at one of the ends,

(b) a microorganism-collecting part located in said hollow container,

(c) a medium for culturing the microorganism, said medium being located so as to contact said microorganism-collecting part during the incubation of the microorganism, and

(d) a disinfectant being located so as to contact the medium for culturing the microorganism after the incubation of the microorganism;

(6) the apparatus for detecting microorganism according to any of the above (1) to (5) wherein an antibiotic substance is added to said medium;

(7) the apparatus for detecting microorganism according to any of the above (1) to (6) wherein an antibiotic substance is contained, at least a part of said antibiotic substance coming unitedly into contact with the medium, by the action of an external force, during the incubation of the microorganism, and at least said antibiotic substance and the medium being located in the storage container in a noncontact manner;

(8) the apparatus for detecting microorganism according to any of the above (1) to (7) wherein microorganisms are detected through incubating the microorganism in a medium and observing the changes in the medium, characterized in that said apparatus comprises

(i) a microorganism-collecting part,

(ii) an antibiotic substance,

(iii) a medium and

(iv) a disinfectant,

wherein at least a part of (i) the microorganism-collecting part, (ii) the antibiotic substance and (iii) the medium can unitedly contact each other, by the action of a force from outside, during the incubation of the microorganism, at least the above-mentioned antibiotic substance (ii) and the above-mentioned medium (iii) being located in a storage container in a noncontact manner, and

wherein, after incubation of the microorganism, at least the incubated microorganism and the disinfectant (iv) can unitedly contact each other by the action of a force from outside, at least the above-mentioned disinfectant (iv) and the above-mentioned medium (iii) being located in the storage container in a noncontact manner;

(9) the apparatus for detecting microorganism according to any of the above (1) to (5) and (7) to (8) wherein said antibiotic substance is

(1) located in the above-mentioned container,

(2) given to the above mentioned microorganism-collecting part or

(3) coated on the inner wall of the above-mentioned container;

(10) the apparatus for detecting microorganism according to any of the above (1) to (5) and (7) to (9) wherein said antibiotic substance is given to other materials located in said hollow container;

(11) the apparatus for detecting microorganism according to any of the above (1) to (5) and (7) to (9) wherein said microorganism-collecting part has a multiple structure containing at least an inside material and an outside material and said antibiotic substance is located in said inside material;



(12) the apparatus for detecting microorganism according to any of the above (1) to (11) wherein said medium contains a substance capable of changing its color according to the change caused by growth of the microorganism;

(13) the apparatus for detecting microorganism according to any of the above (1) to (12) wherein said container has a cover for tight closing and said medium is located in said cover in such a manner that it is enclosed in a bag-shaped member;

(14) the apparatus for detecting microorganism according to the above (13) wherein a perforated material is located between said bag-shaped member and said hollow container;

(15) the apparatus for detecting microorganism according to the above (14) wherein a guide member which can promote a fall of the medium descending along said microorganism-collecting part is located under said perforated material;

(16) the apparatus for detecting microorganism according to any of the above (1) to (15) wherein said apparatus has a incubation selectivity to at least one microorganism selected from the group consisting of pathogenic Escherichia coli, Staphylococcus aureus, Vibrio parahaemolyticus and Salmonella;

(17) the apparatus for detecting microorganism according to any of the above (1) to (16) wherein said apparatus has a structure for allowing us to disinfect the medium used for the incubation under a tightly closed state; and

(18) the apparatus for detecting microorganism according to any of the above (1) to (17) wherein said apparatus has a structure for allowing us to conduct at least

(i) addition of a medium into a container,

(ii) incubation,

(iii) visual detection of the microorganism cells, and

(iv) disinfecting treatment of the incubated medium under a tightly closed state after collecting treatment of the microorganism by the microorganism-collecting part.

The present invention further contemplates:

(19) an apparatus for detecting microorganism, characterized in that,

said apparatus consists of (i) a container having a space wherein a medium is received and the microorganism is cultured and (ii) a cover which engages with an opening of said container to tightly close (fasten or seal) the opening of the container from the surrounding atmosphere (outside),

said cover being a receptacle body having (A) a first bag-shaped member for holding (enclosing) the medium and (B) a second bag-shaped member for holding (enclosing) a disinfectant;

(a) said receptacle body being bestowed with a function for breaking the inner bag-shaped member by the action of a force applied from outside in order to take the content out of said bag-shaped member,

(b) said second bag-shaped member capable of being broken independently of said first bag-shaped member whereby the content in said second bag-shaped member is taken out;

(c) at or near a connecting portion of the cover engaged with the opening of said container, a partition being formed or a partition member being disposed for keeping said bag-shaped member in said cover;

(d) said partition or said partition member being equipped with a microorganism-collecting part which is positioned in a protruding manner at the side of a space of said container for receiving the medium and culturing the microorganism therein;

(e) wherein the end of said microorganism-collecting part can contact said medium in such a manner that it is possible to culture the microorganism when said container receives the medium; and

(f) wherein one or more through holes are formed in said partition or said partition member so that the medium and the disinfectant can flow between said cover and said container;

(20) the apparatus for detecting microorganism according to the above (19), characterized in that,

each of the first and the second bag-shaped members is a glass ampule,

the cover is made of a flexible material and,

even when a force from outside (an external force) is applied to said cover for breaking said first bag-shaped member to take out the medium which is a content therein, said second bag-shaped member is not damaged but the disinfectant which is a content therein can be kept as stored;

(21) the apparatus for detecting microorganism according to the above (19), characterized in that,

the cover is composed of a first section for receiving the first bag-shaped member and a second section for receiving the second bag-shaped member,

said first section being installed with a first pushing-down member for breaking said first bag-shaped member and

said second section being installed with a second pushing-down member for breaking said second bag-shaped member.

(22) the apparatus for detecting microorganism having a structure as shown in any of FIGS. 6 to 11 or characterized in having substantially the same function concerning the bactericidal treatment of the incubated microorganism culture;

(23) An apparatus for detecting microorganism according to the above (19), characterized in that

the cover is composed of:

(a) a first cover-constituting member which constitutes a first section for receiving the first bag-shaped member,

(b) a second cover-constituting member which constitutes a second section for receiving the second bag-shaped member, and

(c) a cap member being located at one end of said second cover-constituting member and at the opposite side of the fitting side (of said second cover-constituting member) for said first cover-constituting member and slidably fitted into said second cover-constituting member,

wherein, at or near each end of the first and second cover-constituting members, each end being at the side of said container having a space for receiving the medium to culture the microorganism therein, a partition is formed or a member for the partition is disposed for keeping each of said bag-shaped members;

said first cover-constituting member being slidably engaged with one end of said second cover-constituting member wherein said first bag-shaped member can be broken to release the medium contained therein by sliding said second cover-constituting member to the side of said first cover-constituting member by a force from outside (external force),

wherein said second bag-shaped member can be broken to release a disinfectant contained therein by sliding said cap member to the side of said second cover-constituting member by a force from outside (external force), and

wherein one or more through holes are formed in said partition or said partition member in a manner that the medium and the disinfectant can be communicated with said container;

(24) the apparatus for detecting microorganism according to the above (23), characterized in that,

an engagement of said first cover-constituting member with one end of said second cover-constituting member is achieved by means of a screw and a force is generated by rotating the main body of said second cover-constituting member against said first cover-constituting member by a force from outside (external force) whereby said first bag-shaped member can be broken to release the medium which is a content therein;

(25) the apparatus for detecting microorganism according to the above (23), characterized in that,

an engagement of said second cover-constituting member with said cap member is achieved by means of a screw and an external force is generated by rotating said cap member against said second cover-constituting member whereby said second bag-shaped member can be broken to release the disinfectant which is a content therein;

(26) the apparatus for detecting microorganism according to the above (24), characterized in that,

the first and second cover-constituting members are constituted from a cylindrical body;

(27) the apparatus for detecting microorganism having an arrangement as shown in FIG. 12 or FIG. 13 or characterized in having substantially the same function concerning the bactericidal treatment of the cultured microorganism;

(28) the apparatus for detecting microorganism according to the above (19), characterized in that,

the cover is composed of:

- (a) a first cover-constituting member which has a first section housing a first bag-shaped member and a second section receiving a second cover-constituting member housing a second bag-shaped member,
- (b) said second cover-constituting member and
- (c) a cap member which is slidably engaged with one end of said second cover-constituting member and is located thereon at the opposite side of the container having a space for receiving said medium to culture the microorganism therein;

wherein, at or near each end of the first and second cover-constituting members, each end being at the side of said container having a space for receiving the medium to culture the microorganism therein, a partition is formed or a member for the partition is disposed for keeping each of said bag-shaped members; said first cover-constituting member being slidably engaged with one end of said second cover-constituting member wherein said first bag-shaped member can be broken to release the medium contained therein by sliding said second cover-constituting member to the side of said first cover-constituting member by a force from outside (external force),

wherein said second bag-shaped member can be broken to release a disinfectant contained therein by sliding said cap member to the side of said second cover-constituting member by a force from outside (external force), and

wherein one or more through holes are formed in said partition or said partition member in a manner that the medium and the disinfectant can be communicated with said container;

(29) the apparatus for detecting microorganism according to the above (28), characterized in that,

an engagement of said first cover-constituting member with a portion of said second cover-constituting member is achieved by means of a screw and a force is generated by rotating the main body of said second cover-constituting member against said first cover-constituting member by a force from outside (external force) whereby said first bag-shaped member can be broken to release the medium which is a content therein;

(30) the apparatus for detecting microorganism according to the above (28), characterized in that,

an engagement of said second cover-constituting member with said cap member is achieved by means of a screw and an external force is generated by rotating said cap member against said second cover-constituting member whereby said second bag-shaped member can be broken to release the disinfectant which is a content therein;

(31) the apparatus for detecting microorganism according to the above (28), characterized in that,

the first and second cover-constituting members are constituted from a cylindrical body;

(32) the apparatus for detecting microorganism according to the above (28), characterized in that;

most of the main body of the second cover-constituting member is housed in the first cover-constituting member;

(33) the apparatus for detecting microorganism as shown in FIG. 14 or characterized in having a function substantially equivalent thereto concerning the bactericidal treatment of the incubated microorganism;

(34) the apparatus for detecting microorganism according to the above (19), characterized in that,

said cover is composed of:

- (a) a receptacle body receiving both of a first bag-shaped member and a second bag-shaped member in parallel and
- (b) two tools for destroying, said tools being slidably engaged with the top of said receptacle body at the opposite side of the container having a space for receiving said medium to culture the microorganism,

wherein said destroying tools are independently pushed into said receptacle body by sliding by means of a force from outside where, first, the first destroying tool can break said first bag-shaped member to release

the medium contained therein and then the second destroying tool can break said second bag-shaped member to release the disinfectant contained therein, and  
 wherein through holes are made in said partition or said partition member so that the medium and the disinfectant can be communicated with the container;

(35) the apparatus for detecting microorganism according to the above (34), characterized in that,

said destroying tools are disposed in such a manner that they can be pushed into the top of said receptacle body by means of a force from outside;

(36) the apparatus for detecting microorganism according to the above (34), characterized in that,

said destroying tools are disposed in such a manner that they can be pushed into the top of said receptacle body in a screwing manner.

(37) the apparatus for detecting microorganism according to the above (34), characterized in that,

the outer appearance of said receptacle body is of a structure similar to a rectangular parallelepiped.

(38) the apparatus for detecting microorganism as shown in FIG. 15 or characterized in having a function substantially equivalent thereto concerning the bactericidal treatment of the incubated microorganism;

(39) the apparatus for detecting microorganism according to the above (19), characterized in that

the cover consists of:

a receptacle body receiving a first bag-shaped member and a second bag-shaped member in parallel, said receptacle body consisting of a holding member which constitutes a holding section capable of regulating the position of said bag-shaped member and a movable material which constitutes a movable section being rotatable by a force from outside against said holding section,

wherein a destroying tool for breaking said bag-shaped member is installed on the inner side of said receptacle body,

wherein, when the movable material is rotated, any of said first bag-shaped member and said second bag-shaped member can be independently broken by said destroying tool, and

wherein through holes are made in said partition or said partition member so that the medium and the disinfectant can be communicated with the container;

(40) the apparatus for detecting microorganism according to the above (39), characterized in that,

a destroying tool is installed on the inner side of the movable material in said receptacle body;

(41) the apparatus for detecting microorganism according to the above (39), characterized in that,

said receptacle body is of a cylindrical shape and,

when the movable material of said receptacle body is rotated, any of said first bag-shaped member and said second bag-shaped member can be independently broken depending upon the direction of rotation; and

(42) the apparatus for detecting microorganism as shown in FIG. 16 or characterized in having substantially the same function concerning the bactericidal treatment of the incubated microorganism.

The present invention further provides:

(43) an apparatus for detecting microorganism, characterized in that

said apparatus comprises:

(a) a hollow container in a cylindrical shape for holding a medium used for culturing the microorganism to be detected during the incubation of the microorganism,

(b) a microorganism-collecting part comprising a rod-like material and a microorganism-collecting end and  
(c) a cover equipped with the microorganism-collecting part wherein said cover stores at least two bag-shaped members where the first bag-shaped member is to receive the medium while the second one is to receive a disinfectant (bactericide), and

wherein (d) the medium enclosed in the first bag-shaped member can get in touch with the said microorganism-collecting end without detaching the cover which closes the opening of said hollow container (a) and the disinfectant/bactericide enclosed in the second bag-shaped member can get in touch with the microorganism in said hollow container to perform a disinfecting/bactericidal treatment;

(44) the apparatus for detecting microorganism according to the above (43), characterized in that,

the cover is constituted from a material which can be easily deformed by a force from outside and the first and second bag-shaped members can be easily broken by a force from outside to discharge the liquid content;

(45) the apparatus for detecting microorganism according to the above (43), characterized in that,

the cover has a partition member at the side of the connecting part with the hollow container and said partition member has an action of receiving and holding the first and the second bag-shaped members in the cover;

(46) the apparatus for detecting microorganism according to the above (43), characterized in that,

the partition member is capable of engaging with said cover at the side of the part (of the cover) connecting with the hollow container; and

(47) the apparatus for detecting microorganism according to the above (43), characterized in that,

the cover is constituted in such a manner that each of the first and second bag-shaped members is independently broken by a force from outside so that the liquid content can be discharged.

The present invention further provides:

(48) the apparatus for detecting microorganism according to the above (19), characterized in that,

said cover is composed of:

- (a) a first cover-constituting member which constitutes a first section receiving and holding a first bag-shaped member,
- (b) a second cover-constituting member which constitutes a second section receiving and holding a second bag-shaped member and
- (c) a cap member which is rotatably screw-fitted at one end of said second cover-constituting member, said cap member being located at the side thereof opposite to the connected side for said first cover-constituting member,


wherein either a partition is formed or a member for the partition is disposed for keeping each of said bag-shaped members at or near the end of each container of the first and the second cover-constituting members having a space for receiving the medium to culture the microorganism therein,

wherein said first cover-constituting member is slidably fixed with one end of said second cover-constituting member and said second cover-constituting member can be slid to the side of said first cover-constituting member by a force from outside so that said first bag-shaped member would be broken to release the medium contained therein,

wherein said cap member can be rotated by a force from outside to push into the side of said second cover-constituting member so that said bag-shaped member would be broken to release a disinfectant contained therein, and

wherein through holes are formed in said partition or said partition member so that the medium and the disinfectant can be communicated with the space;

(49) the apparatus for detecting microorganism according to the above (48), characterized in that,

an engagement of said first cover-constituting member with one end of said second cover-constituting member is secured by means of uneven plane (indented surface: , including a recess and a protruding portion) made on the contacting surfaces between the members and, when the body of said second cover-constituting member is slid by a force from outside to the side of said cover-constituting member, said first bag-shaped member can be broken whereby the medium contained therein can be taken out.

(50) the apparatus for detecting microorganism according to the above (48), characterized in that,

an engagement of said second cover-constituting member with said cap member is in a screw-fixed manner and, when said cap member can be rotated to said second cover-constituting member by a force from outside and pushed into a side of the container whereby said second bag-shaped member would be broken to discharge the disinfectant contained therein;

(51) the apparatus for detecting microorganism according to the above (48), characterized in that,

the first and second cover-constituting members are constituted from a cylindrical body;

(52) the apparatus for detecting microorganism as shown in FIG. 17 or FIG. 18 or that which is characterized in having substantially the same function concerning the bactericidal treatment of the incubated microorganism;

(53) the apparatus for detecting microorganism according to the above (52), equipped with a cap member having the structure as shown in FIG. 19 or FIG. 20 or that which is characterized in having substantially the same function;

(54) the apparatus for detecting microorganism according to the above (52), equipped with a cover-constituting member having the structure as shown in FIG. 22 or FIG. 23 or that which is characterized in having substantially the same function;

(55) the apparatus for detecting microorganism according to the above (52), equipped with a cover-constituting member having the structure as shown in FIG. 26 or FIG. 27 or that which is characterized in having substantially the same function;

(56) the apparatus for detecting microorganism according to the above (48), characterized in that,

one or more convex parts on the side of said first cover-constituting member are formed as a structure having different installing regions, such as 781, 782, and

one or more concave parts suitably engaged with said convex part(s) are formed on the contacting surface of said second cover-constituting members, said contacting surface being contacting the first cover-constituting member,

wherein, before use, it is possible to engage said first cover-constituting member with the second cover-constituting members in such a manner that said concave part is not inserted into said convex part whereby said second cover-constituting member would be fixed within said first cover-constituting member in a nonsliding manner, and, upon use, it is possible to gear said convex part into said concave part by rotating said second cover-constituting member or the like, to ensure an engagement of said first cover-constituting member with one end of said second cover-constituting member by means of concaves and convexes made on the contacting surfaces thereof and, to slide the body of said second cover-constituting member to the side of said first cover-constituting member by means of a force from outside whereby said first bag-shaped member would be broken and the medium contained therein is taken out;

(57) the apparatus for detecting microorganism according to the above (56), characterized in being equipped with a cover-constituting member having the structure as shown in FIGS. 36 to 40 or that which has substantially the same function; and

(58) the apparatus for detecting microorganism according to the above (56), characterized in being equipped with a cover-constituting member having the structure as shown in FIGS. 41 to 44 or that which has substantially the same function.

Still another aspect of the present invention is to provide:

(59) the apparatus for detecting microorganism according to any of the above (1) to (58), comprising a medium selected from the group consisting of:

(a) a medium for Salmonella having a composition substantially containing an appropriate amount, such as 3

to 7 g, of tryptone, an appropriate amount, such as 1 to 6 g, of yeast extract, an appropriate amount, such as 5 to 15 g, of lysine, an appropriate amount, such as 0.5 to 2 g, of glucose, an appropriate amount, such as 7 to 9 g, of sodium chloride, an appropriate amount, such as 1.0 to 2.0 g, of monopotassium dihydrogen phosphate, an appropriate amount, such as 0.1 to 0.3 g, of sodium thiosulfate, an appropriate amount, such as 0.2 to 0.4 g, of ammonium iron citrate, an appropriate amount, such as 15 to 25 g, of magnesium chloride, an appropriate amount, such as 27 to 33 ml, of 0.4% Malachite Green solution and an appropriate amount, such as 0.01 to 0.03 g, of Bromocresol Purple per 1,000 ml of the medium (pH of the medium being about 5.3 to 5.7);

(b) a medium for *Vibrio parahaemolyticus* having a composition substantially containing an appropriate amount, such as 25 to 40 g, of a salt polymyxin broth (the salt polymyxin broth contains an appropriate amount of yeast extract, an appropriate amount of peptone, an appropriate amount of sodium chloride and an appropriate amount polymyxin B), an appropriate amount, such as 15 to 25 g, of mannitol, an appropriate amount, such as 5 to 10 g, of sodium citrate, an appropriate amount, such as 0.1 to 0.3 g, of sodium thiosulfate and an appropriate amount, such as 0.01 to 0.03 g, of Bromocresol Purple per 1,000 ml of the medium (pH of the medium being about 7.0 to 7.4);

(c) a medium for *Escherichia coli* group having a composition substantially containing an appropriate amount, such as 26 to 43 g, of a lauryl sulfate broth (the lauryl sulfate broth contains an appropriate amount of tryptone, an appropriate amount of lactose, an appropriate amount of monopotassium dihydrogen phosphate, an appropriate amount of dipotassium monohydrogen phosphate, an appropriate amount of sodium chloride and an appropriate amount of sodium lauryl sulfate), an appropriate amount, such as 3 to 8 g, of lactose and an appropriate amount, such as 0.03 to 0.05 g, of Bromthymol Blue or Bromocresol Purple per 1,000 ml of the medium (pH of the medium being about 6.75 to 7.25); and

(d) a medium for *Staphylococcus* having a composition substantially containing an appropriate amount, such as 5 to 15 g, of tryptone, an appropriate amount, such as 2 to 8 g, of yeast extract, an appropriate amount, such as 5 to 15 g, of mannitol, an appropriate amount, such as 2 to 8 g, of dipotassium monohydrogen phosphate, an appropriate amount, such as 5 to 6 g, of lithium chloride, an appropriate amount, such as 12 to 20 g, of glycine, an appropriate amount, such as 10 to 14 g, of sodium pyruvate, an appropriate amount, such as 12 to 18 ml, of 1% aqueous potassium tellurite solution and an appropriate amount, such as 0.02 to 0.03 g, of Phenol Red per 1,000 ml of the medium (pH of the medium being about 7.25 to 7.75);

(60) the apparatus for detecting microorganism according to any of the above (48) to (58), characterized in using a medium selected from the improved media described in Example 11; and

(61) the apparatus for detecting microorganism according to the above (1), characterized in that,

after collecting the sample for incubation by means of the microorganism-collecting part, all steps:

- (a) supply of the medium into a container,
- (b) incubation of the microorganism on the medium in the container,
- (c) detection and/or finding of the microorganisms and
- (d) disinfection and/or sterilization of the medium in the container after incubation

can be conducted in a substantially tightly closed system and said apparatus is portable.

Another aspect of the present invention is to provide:

(62) the apparatus for detecting microorganism according to any of the above (1) to (5) wherein microorganisms are detected through incubating the microorganism in a medium and observing the changes in the medium, characterized in that said apparatus comprises

- (i) a microorganism-collecting part,
- (ii) a medium and
- (iii) a disinfectant,

wherein at least a part of (i) the microorganism-collecting part and (ii) the medium can unitedly contact each other, by the action of the force from outside, during the incubation of the microorganism, the above-mentioned microorganism-collecting part (i) and the above-mentioned medium (ii) being at least located in a storage container in a noncontact manner, and

wherein, after incubation of the microorganism, at least the incubated microorganism and the disinfectant



(iii) can unitedly contact each other by the action of the force from outside, at least the above-mentioned disinfectant (iii) and the above-mentioned medium (ii) being located in the storage container in a noncontact manner;

(63) the apparatus for detecting microorganism according to any of the above (1) to (62) wherein said container comprises a cover for closing an opening, said cover accommodating a bag-shaped member which encloses said medium;

(64) the apparatus for detecting microorganism according to any of the above (1) to (63) wherein said container comprises a cover member for closing an opening, said cover accommodating a bag-shaped member which encloses said disinfectant;

(65) the apparatus for detecting microorganism according to any of the above (1) to (64) wherein said cover has a structure for allowing it to hold and carry at least

(i) a first bag-shaped member containing a medium, and

(ii) a second bag-shaped member containing a disinfectant independently each other;

(66) the apparatus for detecting microorganism according to any of the above (1) to (65) wherein said cover comprises at least

(i) a first cover-constituting member accommodating the first bag-shaped member, and

(ii) a second cover-constituting member accommodating the second bag-shaped member;

(67) the apparatus for detecting microorganism according to any of the above (1) to (66), comprising

a means for protection and engagement, being disposed in said first and second cover-constituting members so as not break the first bag-shaped member by moving the second cover-constituting member prior to use;

(68) the apparatus for detecting microorganism according to any of the above (1) to (67), wherein said second cover-constituting member is equipped with a cap member and a means for protection and engagement is disposed in said second cover-constituting member and said cap member so as not to break the second bag-shaped member by moving the cap member prior to use;

(69) the apparatus for detecting microorganism according to the above (67) or (68), wherein said means for protection and engagement is a combination of a stopper (1005, 1008) and a guide (1004, 1007);

(70) the apparatus for detecting microorganism according to any of the above (67) to (69), wherein said guide has a shape selected from the group consisting of shape a, b, c and d as shown in FIG. 70;

(71) the apparatus for detecting microorganism having a structure as shown in any of FIGS. 53 to 64 or characterized in having substantially the same function concerning the bactericidal treatment of the incubated microorganism culture;

(72) a method for quantitatively measuring the number of viable microorganism cells in a sample or specimen through using the apparatus for detecting microorganism according to any of the above (1) to (58) and (60) to (70);

(73) the method according to the above (72), wherein the measurement is carried out in a closed system after collecting a microorganism to be measured and transferring it into the apparatus according to any of the above (1) to (58) and (60) to (70); and

(74) the method according to the above (72) or (73), wherein the target is the number of viable microorganism cells prior to incubation.

The above objects and other objects, features, and advantages of the present invention are readily apparent from the following detailed description of the best mode for carrying out the invention when taken in connection with the accompanying drawings.

#### BRIEF DESCRIPTION OF THE DRAWINGS

Referring to the several drawing figures, wherein like numerals refer to like parts throughout, and wherein:

FIG. 1 is a schematic side cross section showing an embodiment of the microorganism-detecting apparatus of the present invention;

FIG. 2 is a scheme showing an example of how to use the microorganism-detecting apparatus of FIG. 1;

FIG. 3 is a scheme showing an example of how to use the microorganism-detecting apparatus of FIG. 1;

FIG. 4 is a schematic side cross section showing another embodiment of the microorganism-detecting apparatus of the present invention;

FIG. 5 is a schematic side cross section showing another embodiment of the microorganism-detecting apparatus of the present invention;

FIG. 6 shows another embodiment of the microorganism-detecting apparatus of the present invention where the cross-sectional shape of the apparatus is mainly illustrated;

FIG. 7 mainly shows the outer shape of the apparatus of FIG. 6;

FIG. 8 shows the outer shape of the container part supplying a space for incubation of microorganism in the apparatus of FIG. 6;

FIG. 9 shows the cross-sectional structure of the container part supplying the space for incubation of microorganism in the apparatus of FIG. 6 wherein (a) is a shape from its upside while (b) is a shape from its side;

FIG. 10 shows an outer shape of the cover part of the apparatus of FIG. 6 wherein (a) is a shape from its downside, (b) is a shape from its side, and (c) is a shape from its upside;

FIG. 11 shows a cross-sectional structure of the cover part of the apparatus of FIG. 6;

FIG. 12 shows another embodiment of the microorganism-detecting apparatus of the present invention where the outer shape is illustrated;

FIG. 13 shows another embodiment of the microorganism-detecting apparatus of the present invention where the cross-sectional shape of the apparatus of FIG. 12 is illustrated;

FIG. 14 shows another embodiment of the microorganism-detecting apparatus of the present invention where (a) is a ring (77), (b) is a view from the side of the microorganism-detecting apparatus consisting of container and cover, and (c) is a cross-sectional shape of the cover part equipped with (73) and (74);

FIG. 15 shows another embodiment of the microorganism-detecting apparatus of the present invention where (a) is a shape from its upside while (b) is a shape from its side;

FIG. 16 shows another embodiment of the microorganism-detecting apparatus of the present invention where (a) is a cross-sectional shape of the part corresponding to the cover-constituting member (112), (b) is a cross-sectional shape of the part corresponding to the cover-constituting member (102) and (c) is a shape from the side of the microorganism-detecting apparatus;

FIG. 17 shows another embodiment of the microorganism-detecting apparatus of the present invention where the outer shape is illustrated;

FIG. 18 shows a cross-sectional shape of the apparatus of FIG. 17;

FIG. 19 shows an outer shape of a cap member (561) which constitutes the apparatus of FIG. 17;

FIG. 20 shows a cross-sectional shape of a cap member (561) of FIG. 19;

FIG. 21 shows a cross-sectional shape along I-I' of the cross sectional view of FIG. 20;

FIG. 22 shows an outer shape of the cover-constituting member (247) which constitutes the apparatus of FIG. 17;

FIG. 23 shows a cross-sectional shape of the cover-constituting member (247) of FIG. 22;

FIG. 24 shows an outer appearance of the cover-constituting member (247) of FIG. 22 from the direction of II;

FIG. 25 shows an outer appearance of the cover-constituting member (247) of FIG. 22 from the direction of III;

FIG. 26 shows an outer shape of the cover-constituting member (243) which constitutes the apparatus of FIG. 17;

FIG. 27 shows a cross-sectional shape of the cover-constituting member (243) of FIG. 26;

FIG. 28 shows an outer appearance of the cover-constituting member (243) of FIG. 26 from the direction of IV;

FIG. 29 shows an outer appearance of the cover-constituting member (243) of FIG. 26 from the direction of V;

FIG. 30 shows an outer shape of the container (241) which constitutes the apparatus of FIG. 17;

FIG. 31 shows a cross-sectional shape of the container (241) of FIG. 30;

FIG. 32 shows an outer appearance of the container (241) of FIG. 30 from the direction of VI;

FIG. 33 shows a cross sectional shape along G-G' in the cross section of FIG. 31, wherein a cross section of the microorganism-collecting end (246b) is illustrated at its central portion;

FIG. 34 is a scheme of composition of the materials which constitute the microorganism-detecting apparatus of the present invention;

FIG. 35 shows an embodiment of the microorganism-detecting apparatus of the present invention and a cross-sectional shape is shown here;

FIG. 36 shows an outer shape of the cover-constituting member (747) of FIG. 35;

FIG. 37 is a cross-sectional view taken along lines M-M' of the cover-constituting member (747) of FIG. 36;

FIG. 38 shows an outer shape of the cover-constituting member (747) of FIG. 36 from the direction of VII;

FIG. 39 shows an outer shape of the cover-constituting member (747) of FIG. 36 from the direction of VIII;

FIG. 40 shows a cross-sectional shape of the cover-constituting member (747) of FIG. 36;

FIG. 41 shows a cross-sectional shape of the cover-constituting member (743) of FIG. 35;

FIG. 42 shows an outer shape of the cover-constituting member (743) of FIG. 41 from the direction of IX;

FIG. 43 shows an outer shape of the cover-constituting member (743) of FIG. 41 from the direction of X;

FIG. 44 shows a cross-sectional shape of the cover-constituting member (743) of FIG. 41;

FIG. 45 is a cross-sectional view taken along lines N-N' of the cover-constituting member (743) of FIG. 44 ;

FIG. 46 is a cross-sectional view taken along lines P-P' of the cover-constituting member (743) of FIG. 44;  
 FIG. 47 is a cross-sectional view taken along lines Q-Q' of the cover-constituting member (743) of FIG. 44;  
 FIG. 48 is an oblique outer view of the cover-constituting member (743) of FIG. 41 which is made of a transparent (or translucent) stuff; and  
 5 FIG. 49 is a conceptional view showing the relation between the cover-constituting member (743) and that (747) shown in FIG. 35 by showing a small convex ridge (781) and a shallow concave groove (777). This is a state before incubation is started.  
 FIG. 50 is a conceptional view showing the relation between the cover-constituting member (743) and that (747) shown in FIG. 35 by showing a small convex ridge (781) and a shallow concave groove (777). This shows an operation when incubation is started.  
 10 FIG. 51 is a conceptional view showing the relation between the cover-constituting member (743) and that (747) shown in FIG. 35 by showing a small convex ridge (781) and a shallow concave groove (777). This is a state when the cover-constituting member (747) is pushed until a position where an ampule (744) is broken.  
 FIG. 52 shows a structure of a connecting part with a container in a cover-constituting member (e.g., 743) by means of a partial cross section.  
 15 FIG. 53 is a perspective view of an embodiment of the microorganism-detecting apparatus according to the present invention.  
 FIG. 54 illustrates perspectively the assembling of the parts 1245 (and 1561) and 1243 for the microorganism-detecting apparatus of FIG. 53.  
 20 FIG. 55 is a perspective view of an embodiment of the microorganism-detecting apparatus according to the present invention.  
 FIG. 56 illustrates perspectively the assembling of the parts 1245 (and 1561) and 1243 for the microorganism-detecting apparatus of FIG. 55.  
 FIG. 57 is a perspective view of an embodiment of the microorganism-detecting apparatus according to the present invention.  
 25 FIG. 58 illustrates perspectively the assembling of the parts 1561 and 1245 for the microorganism-detecting apparatus of FIG. 57.  
 FIG. 59 is a perspective view of an embodiment of the microorganism-detecting apparatus according to the present invention.  
 30 FIG. 60 illustrates perspectively the assembling of the parts 1561 and 1245 for the microorganism-detecting apparatus of FIG. 59.  
 FIG. 61 is a perspective view of an embodiment of the microorganism-detecting apparatus according to the present invention.  
 FIG. 62 illustrates perspectively the assembling of the parts 1561, 1245 and 1243 for the microorganism-detecting apparatus of FIG. 61.  
 35 FIG. 63 is a perspective view of an embodiment of the microorganism-detecting apparatus according to the present invention.  
 FIG. 64 illustrates perspectively the assembling of the parts 1561, 1245 and 1243 for the microorganism-detecting apparatus of FIG. 63.  
 40 FIG. 65 is a side view of the members (1561, 1245) for an embodiment of the microorganism-detecting apparatus according to the present invention (A: front view and B: side view).  
 FIG. 66 is a side view of the members (1561, 1245) for an embodiment of the microorganism-detecting apparatus according to the present invention.  
 FIG. 67 shows schematically the member 1243 capable of engaging with the member (1245) as illustrated in FIG. 65 (A: top view and B: front side view).  
 45 FIG. 68 is a schematic diagram for explaining the relation between a guide portion (1004) and a stopper (1005) for an embodiment of the microorganism-detecting apparatus according to the present invention.  
 FIG. 69 illustrates schematically the enlarged engagement of a guide portion (1004) with a stopper (1005) at the site B for an embodiment of the microorganism-detecting apparatus according to the present invention.  
 50 FIG. 70 illustrates various shapes for the guides (1004, 1007) for a protecting and engaging means in an embodiment of the microorganism-detecting apparatus according to the present invention.  
 FIG. 71 is a graph showing the relation between the number of viable Staphylococcus aureus cells and measured turbidity data when the microorganism was cultured in an embodiment of the microorganism-detecting apparatus according to the present invention.  
 55 FIG. 72 is a graph showing the relation between the number of viable Escherichia coli cells and measured turbidity data when the microorganism was cultured in an embodiment of the microorganism-detecting apparatus according to the present invention.

Explanation of the symbols (numerals) used is as follows:

- 1, 21, 41, 61, 81, 101, 241 and 741: main body of container
- 2, 22, 42, 62, 82, 102 and 242: cover
- 3: medium
- 4, 24, 44, 64 (or 68), 84, 104, 244 and 744: first bag-shaped member
- 6, 26, 46, 66, 86, 106, 246 and 746: microorganism-collecting member (part)
- 6a, 26a, 46a, 66a, 86a, 106a, 246a and 746a: rod-shaped element (stem)
- 5 and 296: first partition member
- 15 and 235: second partition members
- 6b, 26b, 46b, 66b, 86b, 106b, 246b and 746b: microorganism-collecting end
- 7: disk member impregnated with antibiotic substance
- 9: disinfectant/sterilizer
- 20: guide member
- 11, 59, 79, 89, 109, 259 and 776: connecting part of the container with the cover
- 8, 28, 48, 68 (or 64), 88, 108, 248 and 748: second bag-shaped member

#### BEST MODE FOR CARRYING OUT THE INVENTION

The present invention relates to an apparatus for the detection of microorganisms which is particularly portable or is able to be carried easily, said apparatus being capable of initiating the incubation of the microorganism immediately at the place where the microorganism is collected and/or being capable of starting in the incubation of the microorganism without skillfulness. It is understood that the apparatus for detecting microorganisms in accordance with the present invention includes all things having the function that it is relatively in a small size, portable and, after incubation of the microorganism, able to conduct even the steps until the disposal of the used apparatus in a substantially tightly closed system concerning said incubated microorganism.

The term "substantially tightly closed system" used here means that, although it is possible to incubate, find or detect the microorganism and to disinfect and/or sterilize the incubated microorganism to a disposable extent, the cultured pathogenic microorganism does not diffuse to outside or to environment during the incubating period and/or incubated pathogenic microorganism does not come out to outside or environment by diffusion until the completion of its disinfection and/or sterilization or it does not pollute the outside or environment and the term further means that such a function can be achieved.

In the microorganism-detecting apparatus of the present invention as mentioned above, the medium which is to give an incubating condition suitable for the incubation of the microorganism and the microorganism-collecting part are located in a noncontact manner each other upon the non-using stage while, during the incubation for the detection of the microorganism, those specific medium and microorganism-collecting part are contacted each other whereby the status suitable for proliferation of the microorganism is achieved and, after use, the apparatus can be safely disinfected and/or sterilized.

In the present invention, said medium is further made in an appropriate state where the specific species of the microorganism (food poisoning microorganisms such as *Staphylococcus aureus*) are proliferated but other microorganisms (i.e. those other than *Staphylococcus aureus* such as *Salmonella*, *Escherichia coli*, *Vibrio parahaemolyticus* and other intestinal microorganisms) are substantially unable to grow. After the detection, a treatment for disposal of the apparatus can be conducted by a simple operation and a safe disposal is made possible at all times.

Accordingly, when appropriate incubating means (such as a simple incubator) and detecting means (such as a pH indicator which detects the changes in pH due to growth of the microorganism) of the microorganism are utilized, it is now possible to easily detect the specific species of microorganisms (such as food poisoning ones) and anyone is able to easily dispose the apparatus after use. When an apparatus which is in such a status that it gives an incubating condition having a selectivity to specific species of microorganisms (for example, food poisoning ones such as *Staphylococcus aureus*) is used, it is now easy to selectively detect and identify (i.e. to discriminate the type of the food poisoning microorganism) said specific species of microorganisms (such as food poisoning ones) and to disinfect and sterilize the apparatus containing the pathogenic microorganisms without anxiety.

Examples of the pathogenic microorganism are *Staphylococcus aureus*, *Vibrio parahaemolyticus*, *Salmonella* (such as *Salmonella typhi*, *Salmonella paratyphi* A, *Salmonella schottmuelleri*, *Salmonella enteritidis*, *Salmonella thompson*, *Salmonella narashino*, *Salmonella potsdam*, *Salmonella oranienburg* and *Salmonella senftenberg*), *Clostridium botulinum*, *Shigella* (such as *Shigella dysenteriae* and *Shigella flexneri*), *Vibrio cholerae*, *Vibrio cholerae* biotype *eltor* and *Escherichia coli* (such as enteropathogenic *Escherichia coli* represented by *E. coli* O-157).

When the apparatus is used for detection of food poisoning, the apparatus of the present invention can be preferably made in such a manner that it shows a selective incubating ability to at least one microorganism selected from the

group consisting of Staphylococcus aureus, Vibrio parahaemolyticus and Salmonella which occupy nearly all (about 99%) of the microorganisms causing food poisoning while the discrimination among those types of microorganism may not be essential. This is because, when the presence of at least one of the three types of microorganisms is confirmed, then it is possible to substantially remove all of the three types of microorganisms from the environment wherefrom they are detected if a certain disinfecting means as mentioned later is applied to the environment. When the food poisoning microorganism can be detected and identified in a manner as mentioned above (such as spontaneously), it is now possible to prevent food poisoning easily and effectively by adopting a means suitable for removing said food poisoning microorganism (such as by a disinfecting method where cooking devices, etc. are cleaned with "Hibiden" (trade name: SUMITOMO PHARMACEUTICALS CO. LTD., Japan, for a 0.2 to 0.5% solution of chlorohexidine) or with an invert soap followed by wiping and washing with water).

On the other hand, if it is necessary to identify by discriminating each of Staphylococcus aureus, Vibrio parahaemolyticus, Salmonella and pathogenic Escherichia coli O-157 as a microorganism causing the food poisoning for the sake of the countermeasure, it is also possible that a selective incubating ability is given to the microorganism-detecting apparatus used or a selective identifying ability is given to the detecting function.

If necessary, the apparatus for detecting microorganisms in accordance with the present invention may be constituted in such a manner that a selectivity to incubation of the "big three" food poisoning microorganisms (i.e. Staphylococcus aureus, Vibrio parahaemolyticus and Salmonella; about 99% of the bacterial food poisoning is caused by any of them) is given. It is also possible to give a selectivity to incubation of enterohaemorrhagic Escherichia coli (such as E. coli O-157:H7) which has been an increasingly big problem in recent years. It is further possible to give a selectivity to incubation of antibiotic-resistant microorganisms such as MRSA.

The present invention will now be further illustrated by, if necessary, referring to the attached drawings.

FIG. 1 and FIGS. 3 to 16 show outer appearances, cross sections along the side of the apparatus, etc. of one of the preferred embodiments of the present invention.

First, an illustration will be made by referring to FIG. 1. The detecting apparatus in this embodiment is constituted from a hollow and cylindrical container (1) having an opening at the upper end and a cover (2) which can be freely inserted into and detached from said opening. At the upper part of said cover (2) (i.e. an opposite side to the container opening of the cover (2)), the first bag-shaped member (4) is placed which encloses a medium (3) suitable for the incubation of a specific microorganism such as Staphylococcus aureus. At the upper part thereof, the second bag-shaped member (8) is placed which encloses a disinfectant (9) having a sufficient concentration or amount for disinfecting and/or sterilizing the incubated microorganism. Said medium (3) further contains a pH indicator (such as Phenol Red, Bromcresol Purple and Bromthymol Blue) which identifies the pH changes due to proliferation of the microorganism. On the other hand, at the lower side (an opening side of the container) of said first bag-shaped member (4), a partition member (5) having one or more (preferably, more than one) hole(s) is disposed (e.g. embedded) which prevents falling of the first bag-shaped member (4) into an opening of the container. Further, at the lower side (at the side of the first bag-shaped member) of said second bag-shaped member (4), a partition member (15) having one or more (preferably, more than one) hole(s) is disposed (e.g. embedded) which prevents falling of the second bag-shaped member (8) downward. In order to restrict such a downward movement of the first bag-shaped member (4) and the second one (8), there will be no need of using the partition members (5 and 15) but the structure having the same function, including, for example, an element constituting the cover (2) and having one or more through holes (i.e., a perforated portion in the partition part thereof), will do.

Further, at the nearly central part of the bottom side of the cover (2), a microorganism-collecting member (part) (6) extending to almost vertical direction to the opening side of the above container is placed. Said microorganism-collecting part (6) consists of a rod-like material (stem) (6a) located at the side near the cover (2) and a microorganism-collecting end (6b) located at the end (a far side from the cover (2)) of said rod-like material. Usually, said microorganism-collecting end (6b) is received in such a manner that it does not contact with the medium before use. Said microorganism-collecting end (6b) is to achieve a selective incubation and, usually, said end is received in such a manner that it does not contact with an antibiotic substance having a function of inhibiting the specific microorganism. However, as will be mentioned below in detail, it may be, if necessary, in such a state that some contact with said antibiotic substance is available or that said antibiotic substance is given at said microorganism-collecting end.

Examples of said antibiotic substance are those having a characteristic nature of effectively inhibiting the proliferation of the microorganisms other than specific ones to be detected (e.g., food poisoning microorganisms such as enteropathogenic Escherichia coli and Salmonella and resistant microorganisms such as MRSA). When microorganisms which cause food poisoning (such as Staphylococcus aureus, Vibrio parahaemolyticus and Salmonella) are used as the microorganisms to be detected, examples of the antibiotic substance are aztreonam, polymyxin B, fluconazole and a combination thereof. When antibiotic-resistant microorganism is used as the microorganism to be detected, the examples are oxacillin which inhibits Gram-positive bacteria having no resistance, polymyxin B and aztreonam which inhibits Gram-negative bacteria, polymyxin B as well as a combination thereof.

The antibiotic substance may, as will be mentioned later, be previously added to the medium, coated on the part of

the container (1) used for incubation (such as a bottom or the container (1) or a wall near there), present as a powder which is made to be easily dissolved, or coated at the place which is a part of the microorganism-collecting part (such as the place which is just above the microorganism-collecting end (6b)). It is also possible that said antibiotic substance is placed separately from the part used for incubation in the above-mentioned container, the medium (3) or the microorganism-collecting part (6). In an embodiment where the antibiotic substance is placed separately as such, a disk-shaped member (7) (refer to FIG. 5; preferably composed of "porous material") to which said antibiotic substance is given may, if necessary, be placed at the inner area of the container (1) (such as a space between the microorganism-collecting end (6b) and the bottom of the container). Alternatively, the antibiotic substance may be placed in such a manner that it is supplied when the medium (3) enclosed in the first bag-shaped member (4) (which will be mentioned below) falls down into the container. In such an embodiment, it is also possible that the antibiotic substance is placed at the partition member (5) or at the upper side of the guiding material (20) (cf. FIG. 4).

When a microorganism-detecting apparatus in such an embodiment as shown in FIG. 1 is used, a cover (2) having a microorganism-collecting part (6) is at first taken out from the container as shown in FIG. 2(a). Then, as shown in FIG. 2(b), the microorganism-collecting end (6b) at the end of the microorganism-collecting part (6) is rubbed on an object to be tested (10) (such as a chopping board) and the microorganism existing in said object to be tested (10) is collected to said microorganism-collecting end (6b). Then a cover (2) having the microorganism-collecting part (6) is inserted into the container again and, after that, as shown in FIG. 3, the first bag-shaped member (4) is broken (by, for example, strongly compressing it from outside of the cover (2) using a compressing means which is not shown here) so that the medium (3) which is enclosed and received in said first bag-shaped member (4) is fallen down into the container through the holes of the partition member (5). As a result, the microorganism-collecting end (6b) of the microorganism-collecting part (6) is dipped into said medium (3). At that time, falling of the materials other than the medium such as the broken pieces of the first bag-shaped member (4) is inhibited by the partition member (5) and does not fall down into the container.

For example, as mentioned hereinabove, when an antibiotic substance having a specific selectivity is attached to the microorganism-collecting end (6b), proliferation of the microorganisms other than the specific microorganism (such as food poisoning microorganisms including Staphylococcus aureus and Clostridium botulinum) is substantially inhibited in case the microorganism-collecting end (6b) of the microorganism-collecting part (6) is dipped in the medium (3) as such. Accordingly, when the microorganism-detecting apparatus in such a state is subjected to an incubation by an appropriate incubating means (such as a simple incubator), the specific microorganism (such as food poisoning microorganism including Staphylococcus aureus and Clostridium botulinum) is selectively proliferated. There is no particular limitation for the incubating condition at that time but, usually, the condition of 37 ° C for about 16 to 24 hours is sufficient. After the above-mentioned incubating operation, a change, if any, of the pH indicator contained in the medium (3) (and also the degree of said changes in color) is checked whereby the specific food poisoning microorganism (such as Staphylococcus aureus) can be easily detected and identified (i.e. discrimination of the type of the food poisoning microorganism is conducted). For example, the medium containing Phenol Red as a pH indicator shows a pink color (pH: 7.4) in the initial stage of incubation and, when Staphylococcus aureus is present, it changes to yellow whereby a detection can be done. In addition, physical and chemical changes in the nature and appearance resulted by the substances which are produced by the proliferated microorganism or obtained due to a decomposition thereby, turbidity of the medium and growth of colony are observed as a change in the medium whereby the presence of the microorganism may be judged as well. From a viewpoint of observation by naked eye as such, it is preferred that the container (1) has a light-transmitting ability such as that at least the area wherefrom the observation is conducted is transparent. When there is a possibility that the medium, the antibiotic substance, etc. are affected by light to change their nature, then said transparent area may be in such a constitution that it is covered by a light-shielding coat and, upon observation, it is detached.

Finally, the apparatus where the microorganism is incubated and detected is disposed and, for such a purpose, it is necessary that the incubated cells, cultured products, inner side of the container, etc. are completely disinfected/sterilized. Although not shown in the figures, the second bag-shaped member (8) is broken (by applying a sufficient force for disintegration or by strongly compressing from outside of the cover (2) using, for example, means of disintegrating mechanism or compressing device which is not shown) whereby the disinfectant (9) enclosed and received in said second bag-shaped member (8) is fallen down into the container through the holes of the partitions (5 and 15). As a result, the microorganism-collecting end (6b) of the microorganism-collecting part (6) and the medium (3) containing the incubated microorganism are dipped in and/or mixed with said disinfectant (9). For a complete disinfection/sterilization at that time, it is preferred to shake or the like so that the disinfectant (9) comes throughout the inside of the container (1) and, since a treatment is conducted in a substantially tightly-closed system in the present invention, it is possible to conduct a safe and sure disinfecting/sterilizing operation.

Constitution of each part of the apparatus for detection of microorganisms in accordance with the present invention will be illustrated as follows:

Container

It is preferred that the container is constituted from a transparent or translucent material. To be more specific, the container may be formed using an inorganic material such as glass or an organic material such as plastics (e.g., polyethylene, polypropylene, polystyrene, polyethylene terephthalate (PET), polycarbonate, acrylic resin and polyolefin) and, when weight and possibility of breakage upon centrifugal operation, etc. are taken into consideration, it is preferred that the container is constituted from plastics.

There is no particular limitation for the shape of the container so far as it has an opening at the upper end and a cover (2) can be freely attached to and detached from said opening. To be more specific, in addition to the shape of a test tube as shown in the figures, any of shapes in flask, bottle, etc. may be used.

Cover

There is no particular limitation for the material which constitutes the cover (2) but the material may be the same one as that used for the container. In another embodiment, the cover (2) may be preferably constituted from a material which is softer than that used for the container for lowering the possibility of damage of the container and for increasing the contact adherence with said container. To be more specific, both container and cover may be constituted from polypropylene (PP) or, when the container is constituted from hard plastics or hard resin such as polystyrene and PET, the cover (2) may be constituted from soft plastics such as polyolefin or soft resin (this term is intended to use for covering elastic materials or elastomers as well) or, in some cases, that is rather preferred. A cover is a material having a part placed for connecting to a mouth or a wall of the container. The cover may be in such a form that is fixed to the container by means of either screw or bayonet or is fixed at the certain position by deformation of the connecting part. It is also possible to place a packing or the like at the connecting part of the cover with the container. All or a part of the material which constitutes the cover may be colored for discrimination or may be printed with letters, signs, pictures, etc.

In preferred specific examples, the cap part at the top of the cover may be in different color for each microorganism to be tested so that the type, etc. of the microorganism to be tested can be easily understood and said part may be suitably colored or modified with letters, signs, pictures, etc. It is also possible to use a material which has its color by itself.

In the apparatus of the present invention, it is preferred that the cover (2) can receive at least two bag-shaped members as shown in FIG. 1, FIGS. 4 to 7 and FIGS. 10 to 16 where the first bag-shaped member receives a medium while the second one receives a disinfectant/bactericide.

Bag-Shaped MaterialA. Medium

There is no particular limitation for the stuff, shape, etc. of the first bag-shaped member (4) so far as the first bag-shaped member (4) can surely and stably enclose the medium (3) upon storage and transportation of the microorganism-detecting apparatus and a predetermined amount of the medium (3) can be surely released upon incubation of the microorganism.

To be more specific, stuff for the first bag-shaped member (4) may be a hard stuff such as glass and hard plastics or a soft one such as soft plastics (e.g., polyethylene and polypropylene) and flexible stuff (e.g., paper) but, in view of easiness for confirming the presence of the enclosed medium and the color tone, etc. thereof, it is preferred to use a transparent to translucent material.

In the present invention, there is no particular limitation for the means for releasing the medium (3) from the first bag-shaped member (4) so far as the medium (3) is placed at a position appropriate for incubation upon the incubation of the microorganism. To be more specific, upon releasing the medium (3) from the first bag-shaped member (4), said member (4) may be subjected to any of the means including perforation, cutting and breakage. When perforation, cutting, etc. of the first bag-shaped member (4) is conducted, a material such as a needle-like material or an edge-like material whereby perforation or cutting is made easy (not shown in figures) may be installed at the inner wall of the cover (2) if necessary. On the other hand, in an embodiment where the medium (3) is released by breaking the first bag-shaped member (4), it is preferred in terms of easiness of said breakage that the first bag-shaped member (4) is composed of a hard stuff such as glass and hard plastics. In a structure where a substantially tight-sealed system can be maintained and a sufficient breaking force from outside can be applied to said bag-shaped member, it is possible to easily apply a strong tension to such a hard stuff and to break it whereby the content can be taken out. An example of the preferred structure is that a strong tension is concentrated on a specific area (that may be two or more areas but a considerably narrow area is preferred) of the bag-shaped member made of a hard stuff. As will be mentioned in detail by referring to FIGS. 6 to 16 as hereunder, it is to be understood that such a thing can be achieved by various structures and it is also possible to adopt a structure where known structures and conventional structures are requested to apply



to the microorganism-detecting apparatus and to install that in the microorganism-detecting apparatus of the present invention.

#### B. Disinfectant/Bactericide

In the present invention, there is no particular limitation for stuff, shape, etc. of the second bag-shaped member (8) so far as the disinfectant/bactericide (9) can be safely and surely enclosed and received by the second bag-shaped member (8) during the storage/transportation of the microorganism-detecting apparatus until use and during incubation of the microorganism and so far as a predetermined amount of the disinfectant/bactericide (9) can be surely released upon disposal of the apparatus after use. To be more specific, the stuff for said bag-shaped member can be selected from those which are used for the already-mentioned first bag-shaped member and it is preferably a transparent to translucent material with the purposes that the receiving disinfectant/bactericide (9) can be stably and surely enclosed and that the presence of the predetermined amount of the disinfectant/bactericide can be surely confirmed when the incubated microorganism is sterilized/disinfected or that confirmation of color tone, etc. can be done in an easy manner from a viewpoint of confirmation that the sterilizing/disinfecting treatment was surely done.

In the present invention, there is no particular limitation for the means of releasing the disinfectant/bactericide (9) from the second bag-shaped member so far as the disinfectant/bactericide is positioned to the place suitable for said disinfecting/sterilizing treatment upon the disposing stage of the microorganism-detecting apparatus. To be more specific, it is possible to take any of the means of perforation, cutting, breaking, etc. for the second bag-shaped member (8) in releasing the disinfectant/bactericide (9) from the second bag-shaped member (8). When perforation, cutting, etc. of the second bag-shaped member (8) is conducted, a material such as a needle-like material or an edge-like material whereby perforation or cutting is made easy (not shown in figures) may be installed at the inner wall of the cover (2) if necessary. On the other hand, in an embodiment where the disinfectant/bactericide (9) is released by breaking the second bag-shaped member (8), it is preferred in terms of easiness of said breakage that the second bag-shaped member (8) is composed of a hard stuff such as glass and hard plastics.

In a structure where a substantially tight-sealed system can be maintained and a sufficient breaking force from outside can be applied to said bag-shaped member, it is possible to easily apply a strong tension to such a hard stuff and to break it whereby the content can be taken out. An example of the preferred structure is that a strong tension is concentrated on a specific area (that may be two or more areas but a considerably narrow area is preferred) of the bag-shaped member made of a hard stuff. As mentioned in detail by referring to FIGS. 6 to 16 as hereunder, it is to be understood that such a thing can be achieved by various structures and it is also possible to adopt a structure where known structure and conventional structure are requested to apply to the microorganism-detecting apparatus and to install that in the microorganism-detecting apparatus of the present invention. The force from outside may be that utilizing rotational movement, pushing-in movement, twist, etc.

#### Rod-Like Material

The rod-like material (stem) (6a) constituting the microorganism-collecting part in the present invention is a material having a so-called auxiliary function whereby a microorganism-collecting end (6b) having a function of direct collection of the microorganisms to be tested can be stably supported avoiding a contamination and, in addition, said microorganism-collecting end (6b) is kept at a position suitable for contacting it with the medium (3) upon incubation of the microorganism. There is no particular limitation for stuff, length, shape, etc. for the rod-like material (6a) so far as such functions of a stable holding and of a security for the contacting position are substantially achieved. In terms of prevention of contamination and softness upon collection of the microorganism, it is preferred that said rod-like material (6a) is composed of a flexible plastics.

#### Microorganism-Collecting End

In the present invention, the microorganism-collecting end (6b) constituting the microorganism-collecting part is a material having such functions that it directly collects the microorganism, capable of receiving the collected microorganism in a receiving container without contamination with others after collection of the microorganism and substantially well contacts with the medium (3) during incubation of the microorganism so that a condition suitable for incubation of said collected microorganism is provided. So far as such collecting and contacting functions are substantially achieved, there is no limitation for its structure but any structure will do and there is no particular limitation for stuff, length, shape, etc. of the microorganism-collecting end (6b). To be more specific, said microorganism-collecting end (6b) may be, for example, just an end of the rod-shaped element (6a). However, for an object of easily achieving the above-mentioned collecting and contacting functions, it is preferred that said microorganism-collecting end (6b) has a plane having an increased surface area. Preferred examples of the "plane having an increased surface area" are a surface having one

or more concave(s) (cutting, uneven surface, etc.) (such as an end like ear pick or spatula) and a porous surface. In an embodiment where the microorganism-collecting end (6b) (at least a surface area thereof) is composed of a porous material, a porous material in a form of xerogel or a fibrous material is preferably used. With regard to said fibrous material, filter paper, cotton (such as absorbent cotton), punch felt, cloth, knitted goods, nonwoven fabric, etc. may be used without particular limitation. It is also possible to use a structure where rubber or plastic material is used partially, a hollow structure or a plunger structure using an elastic material or a flexible material or a suckable structure together with a rod-shaped element (6a) which constitute a microorganism-collecting part whereby the sample can be easily collected when it is liquid. Particularly preferred one is that in a shape of an applicator having cotton at its end.

Examples of the object (an object to be tested) wherefrom the microorganism is collected are usually cuisine environment such as utensils of cuisine, cooking devices, etc., home kitchen, toilet, bathtub, the place where food and beverage are sold, etc. Such an object is, for example, wiped with and dipped into the end of the applicator (microorganism-collecting end (6b)) as mentioned above. It goes without saying that, in that case, collection of the sample is usually conducted paying a care that said applicator does not touch with unnecessary part or area. Other examples of the object wherefrom the microorganism is collected are food and food materials.

### Medium

There is no particular limitation for the type, composition, etc. of the above-mentioned medium (3) so far as incubation of the expected microorganism to be detected (such as food poisoning microorganism or resistant microorganism) is possible and known medium for such a microorganism or an improved medium thereof as well as a medium which is modified for better usability may be used. Preferably in the case of food poisoning microorganism, it is possible to suitably select the medium by taking the compatibility (such as reactivity, intersolubility, etc.) with the later-mentioned antibiotic substances, pH indicator, etc. into consideration.

With regard to a medium for Staphylococcus aureus, it is possible to use mannitol-salt (modified) medium, Baird-Parker medium, tellurite-glycine medium, phenylethanol-azide medium, chocolate-agar medium, blood-agar medium, heart infusion agar medium, etc. in the present invention and, in view of the adaptability with the later-mentioned antibiotic substance for selecting Staphylococcus aureus, the use of a mannitol-salt (modified) medium is preferred.

As a medium for microorganism resisting to antibiotics such as MRSA, that which is prepared by adding oxacillin, aztreonam, polymyxin B, or the like to the afore-described medium may be preferably used.

As a medium for Vibrio parahaemolyticus, it is possible to use salt-polymyxin (modified) medium, cellobiose-polymyxin-colistin medium, thiosulfate-citrate-bile salts-sucrose medium, TCBS medium, MacConkey agar medium, blood-agar medium, etc. and, in view of the adaptability with the later-mentioned antibiotics for selecting Vibrio parahaemolyticus, the use of a salt-polymyxin (modified) medium is preferred.

As a medium for Salmonella, it is possible to use xylose-lysine (modified) medium, mannitol-lysine-Crystal Violet Brilliant medium, Salmonella shigella medium, deoxycholate-citrate-lactose-sucrose medium, DHL agar medium, MacConkey agar medium, etc. and, in view of the adaptability with the later-mentioned antibiotics for selecting Salmonella, the use of a xylose-lysine (modified) medium is preferred.

As a medium for Escherichia coli, it is possible to use BTB-lactose-agar medium, blood-agar medium, deoxycholate medium, LB medium, etc.

With regard to such a medium, it is possible to select from those mentioned in known literatures or from those prepared by modifying/varying the above-mentioned ones. Examples of the known literatures are Examined Japanese Patent Publication Hei-07/73,509 (JP Hei 07/73,509 B), Unexamined Japanese Patent Publication (Laid-Open) Hei-01/296,998 (JP Hei 01/296,998 A), etc. for a medium for Vibrio parahaemolyticus; Laid-Open Japanese Patent Publications Sho-52/134,082 (JP Sho 52/134,082 A), Hei-06/217,760 (JP Hei 06/217,760 A), etc. for a medium for Staphylococcus aureus; Laid-Open Japanese Patent Publication Hei-02/65,798 (JP Hei 02/65,798 A), Hei-05/130,859 (JP Hei 05/130,859 A), Hei-06/22,791 (JP Hei 06/22,791 A), etc. for a medium for Salmonella; etc. as well as the references cited therein. In the present invention, a medium which is optionally modified/changed for adapting especially as a liquid medium is preferred.

Usually, a medium in a liquid state is preferably used and that filled or received in a tightly-sealable containers such as ampules or capsules is suitably used although it is not limited thereto but, for example, it may be in such a form that it can be supplied to an incubating space of the container separated by a partition or the like which can be easily broken by a force from outside.

The medium may be an agar medium and, for example, it may be in a form that it does not contact with an antibiotic substance being separated by a partition or the like which can be easily broken by a force from outside. It may be in such a form that, upon incubation, the partition is broken by pushing with an end of the microorganism-collecting rod constituted by a hard material; it is broken by hitting an iron piece or the like coated with glass, plastics or the like placed on said partition by means of a magnet placed outside; or, in addition to the above, a capsule of an antibiotic substance solution or a buffer solution is broken to flow the solution on a medium whereby the microorganism at the collecting end,

the antibiotic substance and the agar medium are contacted. It may also be in such a form that dry medium or powder medium is received therein instead of the agar medium and is reconstituted to the conventional agar medium using an aqueous liquid such as a buffer solution which is received in various forms in the container of the microorganism-detecting apparatus.

In the case of the food poisoning microorganism, it is possible to detect and identify said microorganism by incubating as colonies (by means for a medium in a form of gel, for example). However, in a viewpoint of easiness of detection and identification of the food poisoning microorganism in usual restaurants and home, it is preferred to use a liquid medium giving a homogeneous incubating system to which pH indicator or certain chemical substance capable of acting as an indicator after converted to decomposed product or metabolite as a result of decomposition or metabolism by the microorganism for proliferation of the microorganism is added whereby the judgment can be easily conducted by means of changes in color due to pH change or of naked eye. The medium (3) may preferably be somewhat viscous so far as the detection/identification of the food poisoning microorganism by means of color change is not substantially disturbed.

#### 15 Additives

If necessary, various additives may be added to the above-mentioned medium (3). In an embodiment of the present invention where detection of the microorganism to be detected is confirmed by means of pH change due to proliferation of said microorganism, it is preferred that a pH indicator is previously added to said medium. When such a pH indicator is used, pH of the medium changes as a result of production of acidic substances (such as lactic acid) by proliferation of the microorganism whereby the color tone of the indicator changes and, accordingly, confirmation of said proliferation becomes easy.

There is no particular limitation for the pH indicator which is applicable to the present invention. In view of pH change region, color tone, etc., Phenol Red, Cresol Red, Bromthymol Blue, Bromcresol Purple, triphenyltetrazolium, Blue Tetrazolium, etc. can be appropriately used. Such pH indicators may be used either solely or jointly by combining two or more if necessary. It is also preferred to use each of the pH indicators having different color tones (before changed) in each of the media for detecting different types of microorganism because the microorganism to be detected can be easily recognized at a glance by means of such color tones.

#### 30 Antibiotic Substances

In the present invention, it is also possible that the medium itself is bestowed with some selectivity to the microorganism but, usually, in order to make the selectivity sure, it is preferred that an antibiotic substance which effectively inhibits the proliferation of the microorganism other than the specific one to be detected and identified is jointly used upon necessity. Said antibiotic substance is to achieve a selective incubation of the microorganism to be detected and has a function of inhibiting the proliferation of the microorganism which is other than the specific one to be detected. The type of the antibiotic substance used therefor may be selected depending upon the relation with the microorganism to be detected or may be selected by considering what kind of microorganism selectivity is to be bestowed depending upon the state upon actual use of the apparatus of the present invention.

The "selectivity" upon incubation of microorganism can be adjusted or selected depending upon the purpose. For example, when food poisoning microorganism is an object, it is preferred to have an incubation selectivity to one or more microorganisms selected from the group consisting of "Staphylococcus aureus, Vibrio parahaemolyticus and Salmonella" which occupy nearly all (around 99%) of the microorganisms causing food poisoning while discrimination among those three microorganisms is not essential. However, from a viewpoint of a minute countermeasure to each of the characteristics of those food poisoning microorganisms, it is in some cases preferred to have an "incubation selectivity" whereby discrimination among "Staphylococcus aureus, Vibrio parahaemolyticus and Salmonella" is possible. In addition, there is a case where it is preferred to have an "incubation selectivity" whereby enteropathogenic Escherichia coli such as E. coli O-157 can be discriminatively detected.

In the present invention, there is no particular limitation for the antibiotic substance to be used so far as it is capable of effectively inhibiting the microorganism other than specific (one or more) microorganism(s) to be detected such as food poisoning microorganism. Although the antibiotic substance may be used solely in some cases but, preferably, two or more may be used jointly depending upon the purpose. The amount may be suitably decided especially depending upon the amount of the medium used. For example, in detecting a food poisoning microorganism, the following antibiotic substances are preferably used. In the following description, the concentration for each antibiotic substance is a suitable concentration at the incubating stage of the food poisoning microorganism (when unitedly used with the medium).

( Antibiotic Substances Selective to Staphylococcus aureus )

Aztreonam	1 to 15 µg/ml
Polymyxin B	1 to 15 µg/ml
Fluconazole	1 to 10 µg/ml

Combination of the above three antibiotic substances can be used preferably.

( Antibiotic Substances Selective to Vibrio parahaemolyticus )

Polymyxin B	1 to 15 µg/ml
Fluconazole	1 to 10 µg/ml
Potassium tellurite	1 to 20 µg/ml

Combination of the above three antibiotic substances can be used preferably.

( Antibiotic Substance Selective to Salmonella )

Fluconazole	1 to 10 µg/ml
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#### Locating Position of the Antibiotic Substances

There is no particular limitation for the position or the place where the antibiotic substances are located or placed so far as they function unitedly with the medium (3) at the incubating stage of the microorganism. Preferably, it can be held at a disk member (7) as will be mentioned later in detail (cf. FIG. 5) while other methods are possible as well. Thus, to be more specific, the antibiotic substance may be previously added to a medium (3), coated on an inner wall of the container or bestowed at a certain place of the microorganism-collecting part (6) (the place being able to contact with the medium (3) upon incubation). Two or more of such a positions may be applied if necessary. It goes without saying that, in those cases, the above-mentioned disk member (7) may be omitted. When the antibiotic substance is previously added to a medium (3), it is preferred, by taking inactivation of the antibiotic substance in said medium into consideration, to add the antibiotic substance in such a little bit much amount that said inactivation (usually, a decrease in the antibiotic activity to an extent of around 30 to 35% when stored at ambient temperature for one year) can be supplemented.

When an antibiotic substance is received in a space which is used for incubation in a container, the antibiotic substance may be received in a powdery form but may be received as capsules or tablets which are easily dissolved upon contacting with a liquid such as a medium or, after its solution is added to a position to be used as a space for incubation in the container as mentioned above, it may be received by coating on an inner wall of the container by means of a vacuum drying or the like. Alternatively, the antibiotic substance in a form of an easily soluble powdery or a solution may be filled in a breakable ampule or capsule and received in a space used for incubation in the container in such a manner that it can be easily added. It may be received in any place of the container and may be received in such a manner that it can contact with the medium by shaking the container, by washing out with a liquid medium or by breaking the ampule by a force from outside before incubation. A preferred receiving method is that, before incubation, it keeps a noncontact state with the medium and, for example, the antibiotic substance may be placed at the partition member (5) or above the guide member (20).

On the other hand, in an embodiment where the antibiotic substance is located at a certain position of the microorganism-collecting part (6), it is preferred that the antibiotic substance is located at the rod-shaped member (6a) (the position where contact with the medium (3) is possible) of said microorganism-collecting part (6) and/or at the microorganism-collecting end (6b) which is an end of the microorganism-collecting part (6). When the antibiotic substance is

placed at the microorganism-collecting end (6b), it may be impregnated into said microorganism-collecting end (6b) itself or, after preparing a multi-layered structure consisting of an inner layer (at the side of the rod-shaped element (6a)) which is a microorganism-collecting end (6b) and an outer layer (for collecting the microorganism), the antibiotic substance is placed to said inner layer part. Alternatively, the antibiotic substance may be impregnated in or coated on the upper half area of the microorganism-collecting end (6b).

#### Disk Member

A disk member (7) which is optionally used in the present invention is a material having the functions for holding the antibiotic substance and for offering the condition suitable for incubation of the specific kind of microorganism upon contacting with the medium (3) during the incubation (cf. FIG. 5). There is no particular limitation for the stuff, length, shape, etc. of the disk member so far as such holding and contacting functions are substantially achieved. To be more specific, said disk member may be a simple board or a flat material but, in view of an easy and effective achievement of the above-mentioned holding and contacting functions, it is preferred that said disk member has a plane with an increased surface area. Examples of such "an increased surface area" are a plane with one or more concave(s) (cutting, uneven surface, etc.) and porous surface.

In an embodiment where the disk member (at least its surface area) is composed of a porous material, the preferably used one is a xerogel-like porous material or a fibrous material. Examples of said fibrous material which is applicable without limitation are filter paper, cotton (such as absorbent cotton), punch felt, cloth, knitted goods, nonwoven fabric, etc.

Examples of a medium which does not need a disk member (7) for holding the antibiotic substance, improves the operation ability of the microorganism-detecting apparatus and is suitable as a liquid medium as well are as follows:

#### (a) Composition of a Medium for Salmonella (per 1,000 ml of the medium):

An appropriate amount of tryptone, preferably 3 to 7 g, for instance, 5 g; an appropriate amount of yeast extract, preferably 1 to 6 g, for instance, 3 g; an appropriate amount of lysine, preferably 5 to 15 g, for instance, 10 g; an appropriate amount of glucose, preferably 0.5 to 2 g, for instance, 1 g; an appropriate amount of sodium chloride, preferably 7 to 9 g, for instance, 8 g; an appropriate amount of monopotassium dihydrogen phosphate, preferably 1.0 to 2.0 g, for instance, 1.6 g; an appropriate amount of sodium thiosulfate, preferably 0.1 to 0.3 g, for instance, 0.2 g; an appropriate amount of ammonium iron citrate, preferably 0.2 to 0.4 g, for instance, 0.3 g; an appropriate amount of magnesium chloride, preferably 15 to 25 g, for instance, 20.3 g; an appropriate amount of 0.4% Malachite Green solution, preferably 27 to 33 ml, for instance, 30 ml; and an appropriate amount of Bromocresol Purple, preferably 0.01 to 0.03 g, for instance, 0.02 g

per 1,000 ml of the medium (pH of the medium being about 5.3 to 5.7);

#### (b) Composition of a Medium for Vibrio such as Vibrio parahaemolyticus (per 1,000 ml of the medium):

An appropriate amount of a salt polymyxin broth, for instance, 25 to 40 g (where a salt polymyxin broth contains an appropriate amount of yeast extract, an appropriate amount of peptone, an appropriate amount of sodium chloride and an appropriate amount polymyxin B); an appropriate amount of mannitol, preferably 15 to 25 g, for instance, 20 g; an appropriate amount of sodium citrate, preferably 5 to 10 g, for instance, 8 g; an appropriate amount of sodium thiosulfate, preferably 0.1 to 0.3 g, for instance, 0.2 g; and an appropriate amount of Bromocresol Purple, preferably 0.01 to 0.03 g, for instance, 0.02 g

per 1,000 ml of the medium (pH of the medium being about 7.0 to 7.4);

#### (c) Composition of a Medium for Escherichia coli (per 1,000 ml of the medium):

An appropriate amount of lauryl sulfate broth, preferably 26 to 43 g, for instance, 35.6 g (the lauryl sulfate broth contains an appropriate amount of tryptose, an appropriate amount of lactose, an appropriate amount of monopotassium dihydrogen phosphate, an appropriate amount of dipotassium monohydrogen phosphate, an appropriate amount of sodium chloride and an appropriate amount of sodium lauryl sulfate); an appropriate amount of lactose, preferably 3 to 8 g, for instance, 5 g; and an appropriate amount of Bromthymol Blue or Bromocresol Purple, preferably 0.03 to 0.05 g, for instance, 0.04 g

per 1,000 ml of the medium (pH of the medium being about 6.75 to 7.25); and

#### (d) Composition of a Medium for Staphylococcus such as Staphylococcus aureus (per 1,000 ml of the medium):

An appropriate amount of tryptone, preferably 5 to 15 g, for instance, 10 g; an appropriate amount of yeast extract, preferably 2 to 8 g, for instance, 5 g; an appropriate amount of mannitol, preferably 5 to 15 g, for instance, 10 g; an appropriate amount of dipotassium monohydrogen phosphate, preferably 2 to 8 g, for instance, 5 g; an appropriate amount of lithium chloride, preferably 5 to 6 g, for instance, 5.5 g; an appropriate amount of glycine, preferably 12 to 20 g, for instance, 16.5 g; an appropriate amount of sodium pyruvate, preferably 10 to 14 g, for instance, 12 g; an appropriate amount of 1% aqueous potassium tellurite solution, preferably 12 to 18 ml, for instance, 15 ml; and an appropriate amount of Phenol Red, preferably 0.02 to 0.03 g, for instance, 0.025 g

per 1,000 ml of the medium (pH of the medium being about 7.25 to 7.75).

Among the components for the medium, amounts of those which are added as nutrients such as tryptone, yeast extract, lysine, glucose, peptone, mannitol, tryptose and lactose may be either increased or decreased by persons skilled in the art depending upon the object and necessity and it is to be understood that such a medium is also included in the coverage of the medium of the present invention. Examples of the components related to the selectivity of the microorganism to be detected are sodium chloride, Malachite Green, Bromcresol Purple, sodium thiosulfate, ammonium iron citrate, polymyxin B, sodium lauryl sulfate, Bromthymol Blue, lithium chloride, sodium pyruvate, potassium tellurite and Phenol Red. Since such components have considerable affection on the sensitivity for the microorganism detection, it is requested that the adding amounts are precisely selected. However, it is also acceptable to select the amounts which are out of the above-mentioned ranges after conducting normal tests or the like by incubating the microorganism known in the art and selecting and deciding the due amounts. It is to be understood that the medium with such a modification or improvement is also within a coverage of the present invention.

Those media are liquid ones having a fluidity and are particularly suitable for utilization in the microorganism-detecting apparatus of the present invention. Those media show excellent microorganism-detecting sensitivity and specificity to the microorganism and, at the same time, they have an excellent detectability to the microorganism due to color change of the pH indicator. The amounts of the components constituting the above-mentioned media may be either increased or decreased by a person skilled in the art and it is also possible to add appropriate component(s) provided that the basic characteristics are unaltered. Commercially-available ones may be used as the components to be compounded with the medium. For example, salt-polymyxin broth is available from NISSUI SEIYAKU K.K., Japan, lauryl sulfate broth is available from DIFCO and other components are available from DIFCO, WAKO PURE CHEMICAL INDUSTRIES, LTD., etc.

The apparatus of the present invention for the detection of microorganisms is useful for detecting specific kinds of microorganism (food poisoning microorganisms such as Staphylococcus aureus, Salmonella, Escherichia coli, Vibrio parahaemolyticus and other intestinal microorganisms) in cooking environment (such as equipment and utensil at cuisine) and human living environment including kitchen, toilet, bath room and shops where food and beverage are sold. It is also possible to utilize the microorganism-detecting apparatus of the present invention with an object of detecting the afore-described microorganisms in food and food materials containing various components in high concentrations such as for detecting the contamination by food poisoning microorganism. Food and food materials contain various components such as saccharides, proteins, lipids, organic acids (e.g., acetic acid and citric acid), amino acids, chemical condiments, vitamins, minerals and other chemical substances. As a result thereof, pH, ion strength, etc. of the medium change and, when detection is conducted where the principle of the detection utilizes the color change of the pH indicator for the pH change of the medium due to proliferation of microorganism, there is a possibility that the above changes in pH and ion strength affect on the pH indicator used there. In that case, it is possible to prepare an appropriate sample depending upon the type of the food and food materials to be tested by, for example, subjecting the sample to a pretreatment (such as adjustment of pH), selecting an appropriate pH indicator, changing the components of the medium or changing the amounts of the components. For example, when the food to be tested is beverage such as a milk product and an object is to detect Escherichia coli, Bromthymol Blue used as a pH indicator may be substituted with Bromcresol Purple.

#### Aseptic Condition

The microorganism-detecting apparatus of the present invention as mentioned above is preferably in an aseptic condition (at least in its inner area) immediately before the use in view of correctness of the test. In making the apparatus of the present invention aseptic after its manufacture, known physical means (e.g., sterilization by irradiation of electromagnetic waves such as ultraviolet ray and gamma-ray) and/or chemical means (e.g., sterilization by gas such as ethylene oxide) may be used without particular limitation. For example, the apparatus can be sterilized by irradiating with gamma-ray at 1 to 30 ° C for one minute. When sterilization is conducted by a sterilizer using ethylene oxide, sterilization can be achieved by conducting at, for example, 0 to 70 ° C (preferably, at 30 to 50 ° C) in a gas consisting of about 10 to 40% of ethylene oxide and about 60 to 90% of carbon dioxide gas (preferably, about 20% of ethylene oxide and about 80% of carbon dioxide gas) under certain pressure (preferably, at the pressure of about 0.1 to 1 kgw/m<sup>2</sup> or, more preferably, at about 0.3 kgw/m<sup>2</sup>) for about 1 to 48 hours (preferably, about 3 to 10 hours).

After the above-mentioned sterilization, it is preferred that the microorganism-detecting apparatus of the present invention is stored in a bag which is made of film or the like (not shown). At that time, the microorganism-detecting apparatus (FIG. 1) may be unitedly stored in the same bag or, if necessary, each of the container and the cover (2) (and the microorganism-collecting part (6)) may be stored in different endosures.

With regard to selection and combination of the afore-described microorganism-collecting end, various receiving forms concerning the antibiotic substances and various forms concerning the medium, a lot of variations and combina-

tions are allowable provided that they are not out of the objects and characteristic features of the present invention. Especially in the case where both (or at least one of) antibiotic substance and medium (particularly preferably, liquid medium) are/is received in a bag, ampule or capsule made of synthetic resin or glass and, upon actual use, said receiver is broken whereby the antibiotic substance and the medium are unitedly combined, it is preferred that the area of the container receiving said bag, ampule or capsule is made of a material or in a shape whereby such bag, ampule or capsule can be easily broken or destroyed by a breaking device or by a force from outside without such a device. For example, the area is flexible or is a substance having elasticity or flexibility.

#### Disinfectant/Bactericide

The disinfectant or bactericide which is used for disinfecting and/or sterilizing the incubated pathogenic microorganism in the microorganism-detecting apparatus after use whereby said used microorganism-detecting apparatus can be safely disposed is added in such a state that substantially separated from outside or from environment since the incubation of the microorganism to be tested is started so that the area containing the incubated microorganism can be disinfected and/or sterilized. If it is in such a structure, it may be located in the microorganism-detecting apparatus of the present invention in any form but a representative example is that disinfectant/bactericide is present in a form that it is received in the second bag-shaped member (8) which is different from the first bag-shaped member for receiving the medium (3) in a cover (2). With regard to the disinfectant/bactericide, one which is suitably selected from disinfectants and/or bactericides which have been known in the art may be used and the examples are disinfectants/bactericides of a chlorate type, those of an invert soap type, those of a biguanide type, those of an aldehyde type and those of a phenol or cresol type. Representative examples of the disinfectant/ bactericide are sodium hypochlorite, bleaching powder, chloramine-T, benzalkonium chloride, benzethonium chloride, chlorhexidine salt, polyalkylene biguanidine salt, formaline, glutaraldehyde and pompidon iodine.

The disinfectant (bactericide) used may be selected from those which have been commonly used for disinfecting, sterilizing or washing the area wherefrom pathogenic microorganism such as food poisoning microorganism was detected such as chopping board and cooking board or which have been found to be preferred for such an object. Examples of such a detergent (disinfectant) are the above-mentioned sodium hypochlorite (WAKO PURE CHEMICAL INDUSTRIES, LTD., Japan), "Tego-51" (10%; trade name of NIPPON SHOJI K.K., Japan), "Kitchen Kabi-Killer" (trade name of JOHNSON K.K., Japan) and "Kitchen Haitei" (trade name of KAO K.K., Japan).

Such a detergent (disinfectant) may also be that where its disinfecting effect has been well noted by the following test method using, for example, Staphylococcus aureus ATCC 25923, Escherichia coli ATCC 25922, Enterobacter faecalis ATCC 29212 and Pseudomonas aeruginosa ATCC 27853.

#### Method for Testing the Disinfecting (Sterilizing) Effect

##### (1) Preparation of Microorganism Solution

Standard strains of Staphylococcus aureus ATCC 25923, Escherichia coli ATCC 25922, Enterobacter faecalis ATCC 29212 and Pseudomonas aeruginosa ATCC 27853 incubated for 24 hours were used and prepared into a solution having a McFarland's standard turbidity of No.1 ( $3.0 \times 10^8$  CFU/ml) using a sterile physiological saline solution.

##### (2) Sterilizing Method

Each of the washings was used as an original solution and contacted, in a final concentration of 10%, with for five minutes with the microorganism solution prepared in the above (1).

##### (3) Incubating (Culturing) Method

The microorganism solution contacted with the washing for five minutes as mentioned in the above (2) was used as an original solution, diluted to 10-fold (x10) and 100-fold (x100) with a sterile physiological saline solution and each 0.1 ml of them was inoculated to a blood-agar medium followed by incubating. As a control, a system containing no detergent was prepared and incubated by the same manner as above. Numbers of the colonies grown after incubating at 37 ° C for 48 hours were compared with those of the control whereby the disinfecting effect was evaluated.

When sodium hypochlorite (WAKO PURE CHEMICAL INDUSTRIES, LTD., Japan), "Tego-51" (10%; trade name of NIPPON SHOJI K.K., Japan), "Kitchen Kabi-Killer" (trade name of JOHNSON K.K., Japan) and "Kitchen Haitei" (trade name of KAO K.K., Japan) were used as detergents, all of the standard strains were found to be sterilized within five minutes by those detergents.

FIG. 4 shows another embodiment of the microorganism-detecting apparatus of the present invention. In this



embodiment of FIG. 4, a "guide member" (20) has a function of promoting the falling-down of a medium (3) along the rod-shaped element (6a) when the medium (3) falls down into a container (1) and said guide member is disposed at the lower area of the partition member (5). Constitution of FIG. 4 is the same as that of FIG. 1 except that said guide member (20) is installed therein.

There is no particular limitation for the stuff, shape, size, etc. of the guide member (20) so far as said guide member (20) plays a function of promoting the falling-down of the medium (3) along the rod-shaped element (6a). In view of the easiness in promoting the falling-down of the medium (3) along the rod-shaped element (6a), it is preferred that the guide member (20), at least a part thereof, has a smaller inner diameter than that of the container (1).

To be more specific, it is preferred that, as shown in FIG. 4, said guide member (20) is composed of a "fan"-shaped member where the lower end of the guide member is somewhat inclined to the central direction of the container (1) (i.e., in other words, said lower end has a smaller diameter than the inner diameter of the container (1)) although such an "inclination" may be omitted. FIG. 5 shows a microorganism-detecting apparatus of the present invention where the disk member (7) is placed in the container (1).

FIGS. 6 to 11 are to show a basic constitution of the microorganism-detecting apparatus of the present invention in another representative embodiment, particularly to show a constitution basically bestowed as a means for disinfecting and/or sterilizing the cultured medium, etc. after incubation. FIG. 6 mainly shows a cross sectional view of said microorganism-detecting apparatus; FIG. 7 mainly shows an outer appearance of said microorganism-detecting apparatus; FIG. 8 shows an outer appearance of the container (21) for said microorganism-detecting apparatus; FIG. 9a shows a view from the upper side of the container (21) for said microorganism-detecting apparatus; FIG. 9b shows a cross sectional view of the side of the container (21) for said microorganism-detecting apparatus; FIG. 10a is a shape of the cover (22) for said microorganism-detecting apparatus and is the shape observed from the connected side with the container (21); FIG. 10b mainly shows an outer appearance from one side of the cover (22) for said microorganism-detecting apparatus; FIG. 10c is a shape of the cover (22) for said microorganism-detecting apparatus and shows the view from the upper part (from the side of the cap (261)); and FIG. 11 shows the cross sectional view from the side of the container for said microorganism-detecting apparatus (the microorganism-collecting part (member) is omitted in FIG. 10 and FIG. 11).

In FIG. 6, the cover (22) is mainly composed of (i) a cover-constituting member (housing) (23) capable of receiving an ampule (24) where a medium is contained, (ii) a cover-constituting member (housing) (27) capable of receiving an ampule (28) where a disinfectant is contained, and (iii) a cap member (261). A protrusion (or projection) (32) is disposed at the bottom side of the cover-constituting member (23), i.e. at the connecting side with the cylindrical container (21). The protrusion (32) may be placed throughout the circumference of the cover-constituting member (23) whereby the connecting side portion of the cover-constituting member (23) with the container (21) becomes thick. Alternatively, the protrusion (32) may be sporadically placed in a projecting manner on the inner side of the cover-constituting member (23) such as a projection of a crosspiece (sash bar) or the like. In a preferred embodiment, a screw cutting is done at the top of the cover-constituting member (23), i.e. at the end (34) at the side of the cap-member (261) as to engage with the screw threads made on the cover-constituting member (27) and, when the main body of the cover-constituting member (27) is rotated, a bottom (35) of the area where the ampule (28) in the cover-constituting member (27) is received can be pushed against the side of the container (21). Alternatively, the top of the cover-constituting member (23), i.e. an end (34) at the side of the cap member (261), is tightly engaged with an end of the cover-constituting member (27) at the side of the container (21) and, when the cover-constituting member (27) is pushed by sliding to the side of the container (21), the bottom (35) of the ampule (28)-receiving area of the cover-constituting member (27) can be easily pushed against the side of the container (21) whereby the ampule (24) can be pushed against the side of the container (21).

In the cover-constituting member (27), there are through holes at the bottom (35) of the receiving part for ampules (28) so that the disinfectant in the ampule flows down into the container (21) when the ampule (28) is broken. Like in the cover-constituting member (23), there are protrusions (or projections) (33) at the bottom side of the cover-constituting member (27), i.e. at the side of the ampule (24). Like in the afore-mentioned protrusions (32), the protrusions (33) may be placed throughout the circumference of the cover-constituting member (27) whereby the bottom (35) of the ampule-receiving part of the cover-constituting member (27) becomes thick at the side of the ampule (24). Alternatively, the protrusions (33) may be sporadically placed in a projecting manner on the inner side of the cover-constituting member (27). In a preferred embodiment, a screw cutting is done at the top of the cover-constituting member (27), i.e. at the end (37) at the side of the cap-member (261) so as to engage with the screw threads made on the cover-constituting member (261) and, when the head of the cap member (261) is rotated, a bottom (36) of the cap member (261) can be pushed against the side of the ampule (28). Alternatively, the top of the cover-constituting member (27), i.e. an end (37) at the side of the cap member (261), is tightly engaged with the cap member (261) and, when the cap member (261) is pushed into by sliding to the side of the container (21), the bottom (36) of the ampule (28)-receiving area of the cover-constituting member (261) can be easily pushed against the side of the container (21) whereby the ampule (28) can be pushed against the side of the container (21).

First, when the main part of the cover-constituting member (27) is rotated (or pushed against the container side) and the bottom (35) of the receiving area for the ampule (28) in the cover-constituting member (27) is pushed against the side of the container (21), then said bottom (35) pushes down the ampule (24). Such a pushed-down ampule (24) is then supported with the slope of the protrusion (32) disposed on the cover-constituting member (23). As such, when the cover-constituting member (27) is pushed down to the side of the container (21), a tension would occur at a contacting point (A) of the ampule (24) with the slope of the protrusion (32) whereby the lower part of the ampule (24) is broken and the content therein is allowed to be released. Said content coming out from the ampule (24) flows down into a container (21) due to gravity and contacts with the microorganism-collecting end (26b). (Incidentally, in FIG. 6, through holes, attaching area of the microorganism-collecting part, fine structure of joint portions between the cover and the container, etc. are omitted.)

During the disinfection after incubation of the microorganism, when the top of the cap member (261) is rotated (or pushed against the side of the container) and the bottom (36) of the cap member (261) is pushed against the side of the ampule (28), then the bottom (36) of the cap member (261) pushes down the ampule (28). The pushed-down ampule (28) is then supported on the slope of the protrusion (33) placed at the cover-constituting member (27). As such, when the cap member (261) is pushed against the side of the container (21), then a tension would occur at a contacting point (B) of the ampule (28) with the slope of the protrusion (33) whereby the lower part of the ampule (28) is broken and the content therein is allowed to be released. The content coming out from the ampule (28) flows down into a container (21) through the perforations placed at the bottom (35) of the ampule (28)-receiving part of the cover-constituting member (27) due to gravity and contacts with the incubated microorganism. It is preferred in view of the actual use that the ampule is made of fragile material such as glass or hard plastics and that, when it is applied with a mechanical force (particularly when the mechanical force is partly applied), the ampule is easily broken.

The medium containing the incubated pathogenic microorganism and the space in the container where there is a high possibility of contamination with the microorganism are disinfected and/or sterilized by a disinfectant. As such, the apparatus can be made into a safely disposable state. It is possible for the incubation system of the apparatus of the present invention that, since the beginning of incubation by supplying the medium into an apparatus (1), certain microorganisms (targets) are found/detected by observing the incubated medium and, at last, the apparatus is disinfected and/or sterilized to make it disposable while said incubation system is kept in a substantially tightly closed state to outside or to environment throughout the above steps. The microorganism-detecting apparatus of the present invention is useful with a view that, even if the target microorganism to be tested is not limited to pathogenic but ordinary (nonpathogenic), pollution of environment (or possibility thereof) is to be avoided. Further, the microorganism-detecting apparatus has an excellent portability and, therefore, the fact that said apparatus can be disinfected/sterilized to such an extent that it can be safely and surely disposed is valuable and appreciated. In addition, it is clear from the structure of the apparatus of the present invention that said microorganism-detecting apparatus can be surely, easily and securely operated even by unskillful persons.

FIG. 12 and FIG. 13 show a constitution which is fundamentally present in another representative embodiment of the microorganism-detecting apparatus of the present invention, particularly with a means for disinfecting/sterilizing the cultured medium, etc. after incubation.

In FIG. 13, a cover (42) is mainly composed of (i) a cover-constituting member (43) capable of receiving an ampule (44) containing a medium, (ii) a cover-constituting member (47) capable of receiving an ampule (48) containing a disinfectant, and (iii) a cap member (49). At the bottom side of the cover-constituting member (43), i.e. at the side of the junction (59) with the cylindrical container (41), there is a protruding portion (or projection) (52). The protrusion (52) may be placed throughout the circumference of the cover-constituting member (43) whereby the cover-constituting member (43) becomes thick at the engaging side (59) thereof with the container (41) or the protrusion (52) may be sporadically placed in a projecting manner on the inner side of the cover-constituting member (43), such as a protrusion of a cross-piece or the like. In a preferred embodiment, a screw cutting is done at the top of the cover-constituting member (43), i.e. at the end (54) at the side of the cap member (49) so as to engage with the screw threads made on the cover-constituting member (47) and, when a head part (45) of the cover-constituting member (47) is rotated, a bottom (55) of the cover-constituting member (47) can be pushed against the side of the container (41). The bottom part (55) of the cover-constituting member (47) has one or more through holes whereby, when an ampule (48) is broken, the disinfectant contained therein can flow into a container (41) as mentioned below (incidentally, in FIG. 13, the through hole is not shown).

A protruding portion (or projection) (53) is disposed at the bottom side of the cover-constituting member (47), i.e. at the side of an ampule (44), like the cover-constituting member (43). The protrusion (53) may be disposed, like the above protrusion (52), throughout the circumference of the cover-constituting member (47) whereby the cover-constituting member (47) becomes thick at the bottom side (55) of the ampule side (44). Alternatively, the protrusion (53) may be sporadically placed in a projecting manner on the inner side of the cover-constituting member (47). In a preferred embodiment, a screw cutting is done at the top of the cover-constituting member (47), i.e. at the end (57) at the side of the cap member (49) so as to engage with the screw threads made on the cap member (49) and, when a head part of the cap member (49) is rotated, a bottom (56) of the cap member (49) can be pushed against the side of the ampule

(48).

First, when a head part (45) of the cover-constituting member (47) is rotated so that the cover-constituting member (47) is pushed against the side of the container (41), a bottom (55) of the cover-constituting member (47) pushes down the ampule (44). The pushed-down ampule (44) is then supported by a slope of the protrusion (or projection) (52) disposed in the cover-constituting member (43). As the cover-constituting member (47) is pushed further against the side of the container (41), a tension becomes higher at the contacting portion (A) between the ampule (44) and a slope of the protrusion (52) whereupon the lower area of the ampule (44) is broken and the content therein is allowed to release. The content coming out from the ampule (44) flows down into the container (41) because of the force of gravity and comes in contact with the microorganism-collecting end (46b). (Incidentally, in FIG. 13, a through hole at (59), a connecting area for the microorganism-collecting member, the detailed structure of the engagement between the cover and the container, etc. are omitted.)

At the disinfecting stage after incubation, when the bottom (56) of the cap member (49) is pushed against the side of the ampule (48) by rotating the top portion (461) of the cap member (49), a bottom (56) of the cap member (49) pushes down the ampule (48). The pushed-down ampule (48) is then supported by a slope of the protrusion (53) disposed in the cover-constituting member (47). As the cap member (49) is pushed further against the container (41) side, a tension would occur higher at the contacting area (B) of the ampule (48) with the slope of the protrusion (53) whereupon the lower part of the ampule (48) is broken and the content therein is allowed to release. The content coming out from the ampule (48) flows down by gravity into a container (41) through a perforated hole (through hole) (not shown) formed in the bottom (55) of the cover-constituting member (47) and comes in contact with the incubated microorganism. It is preferred in terms of actual use that the ampule is made of fragile material including glass, hard plastic material, etc. whereby it can be easily broken upon application (especially, partial application) of mechanical force.

The medium containing the incubated pathogenic microorganisms, etc. and the space of the container having a high possibility of contamination with the microorganisms are disinfected and/or sterilized by a disinfectant. As such, the apparatus can be made safely disposable. It is possible for the incubation system of the apparatus of the present invention that, since the beginning of incubation by supplying the medium into an apparatus (1), certain microorganism is found/detected by observing the incubated (cultured) medium and, at last, the apparatus is disinfected/sterilized to make it disposable while said incubation system is kept in a substantially tightly closed state to outside (surrounding atmosphere) or to environment throughout the above steps. The microorganism-detecting apparatus of the present invention is useful and characteristic with a view that the microorganisms to be tested is not limited to pathogenic ones and pollution of environment (or possibility thereof) is to be avoided even if the microorganisms to be tested are ordinary ones. Further, the microorganism-detecting apparatus has an excellent portability and an unique arrangement and, therefore, the fact that said apparatus can be disinfected/sterilized to such an extent that it can be safely and surely disposed is valuable and highly appreciated. In addition, it is clear and apparent from the arrangement of the apparatus according to the present invention that said microorganism-detecting apparatus can be surely, securely and readily operated even by unskillful persons.

FIG. 14 shows a constitution which is fundamentally present in one representative embodiment of the microorganism-detecting apparatus of the present invention, like those as shown in FIG. 12 and FIG. 13. It uniquely has a means for disinfecting and/or sterilizing the cultured medium, etc. after incubation.

In FIG. 14, the cover is mainly composed of a cover-constituting member (62) which is capable of receiving and holding (i) an ampule containing a medium and (ii) an ampule containing a disinfectant. At the side contacting with the surface of ampules (64/68), protrusions (or projections) (73 and 74) are installed in a form of grooves (or of ridges) on the inner side of the cover-constituting member (62). Refer, for example, to FIG. 14(c). When a ring (77) (cf. FIG. 14(a)) having a slightly smaller diameter than a diameter (or outer diameter) of the cover (62) accommodating the ampule is prepared and passed from a top (71) of the cover (62) to the side of a container (61), said ring (77) smoothly passes at the area of (72) in the cover since there is no projection there but, after the area (72), tension due to the protrusions (73 and 74) is applied to the ampule and forces the ampule to be broken from its side. When the protrusions (73 and 74) are made to come closer to the ampule upon coming nearer the container (61), then stronger tension bears on the ampule as the ring (77) passes from the top (71) of the cover (62) to the side of the container (61). Incidentally, in FIG. 14, a perforated hole (through hole) for the medium and the disinfectant each coming out from each ampule (64/68) into a container (61), an area where the microorganism-collecting part is attached, the detailed arrangement of engaging portion between the cover and the container, etc. are omitted.

In another embodiment, the cover is mainly composed of a cover-constituting member (62) capable of receiving and holding (i) an ampule (64) containing the medium and (ii) an ampule (68) containing the disinfectant as same as that mentioned hereinabove. Alternatively, it is also possible that a ring (77) is previously installed on the area (76) of the cover (62) at the container (61) side and the ring (77) is raised to the top (71) side of the cover (62) whereby breaking is started from the lower ampule (64) as same as above.

In still another embodiment, the cover is mainly composed of a cover-constituting member (62) capable of receiving and holding (i) an ampule (64) containing a disinfectant and (ii) an ampule (68) containing the medium. A ring (77) pre-

pared already is passed to a container (61) from the top (71) of the cover (62) so that the ampule (68) is broken from its side. The ring (77) which was moved for breaking the ampule (68) as such is then moved to the middle portion of the cover (62) and either stopped there or moved upward followed by taking away from the top (71). For breaking another ampule (64), a ring (77) (hereinafter, referred to as (77')) (not shown) is previously installed on the area (76) of the cover (62) at the side of the container (61) and said ring (77') is raised to the top side (71) of the cover (62) whereby the ampule (64) is broken from the lower part. Alternatively, it is also possible that the cover is mainly composed of a cover-constituting member (62) capable of receiving and holding (i) an ampule (64) containing the medium and (ii) an ampule (68) containing the disinfectant and that the ring (77) and the ring (77') are moved in a reversed order as compared with the above, whereby the medium is first supplied to the container (61) and, after incubation, the disinfectant is supplied to the container (61).

FIG. 15 illustrates a fundamental arrangement required in a representative specific embodiment of the microorganism-detecting apparatus according to the present invention, particularly the fundamental arrangement for disinfecting and/or sterilizing the cultured medium, etc. after incubation.

In FIG. 15, the cover (82) is mainly composed of a cover-constituting member capable of receiving and storing (i) an ampule (84) containing a medium and (ii) an ampule (88) containing a disinfectant. In the cover-constituting member (82), there are pushing-down members (91 and 93) at the top (99) which is an opposite side to the container (81). Said pushing-down members (91) is equipped with a part (92) having a shape for allowing a force to be applied to the top of the ampule (84) in a stable manner. Similarly, another pushing-down member (93) is equipped with a part (94) (in the drawing, this symbol is omitted) having a shape for allowing a force to be applied to the top of the ampule (88) in a stable manner.

First, when the pushing-down member (91) is pushed in, the ampule (84) is pushed down via the part (92). Then tension would occur at a contacting point (95) of the ampule (84) with a slope (or crosspiece-like projection) (not shown) formed at the side near the container (81) and on the inner side (ampule-receiving side) of the cover (82). Said tension forces the lower part of the ampule to be broken whereby the medium is taken out from the ampule and comes into the container (81) by gravity through one or more perforated holes (through hole) (not shown) in the engaging portion (89). When another pushing-down member (93) is pushed in after incubation of the microorganism, the ampule (88) is pushed down via the part (94) whereupon a tension would occur at a contacting point (96) of the ampule (88) with a slope (or crosspiece-like projection) (not shown) located at the side near the container (81) and on the inner side (ampule-receiving and storing side) of the cover (82) and forces the lower part of the ampule to be broken whereby the disinfectant is released from the ampule and comes into the container (81) through the through hole (perforated hole) (not shown) of the engaging portion (89) by gravity.

The pushing-down members (91/93) may be installed at the top (99) of the cover (82) in such a manner that they can be pushed in by a force from outside which is more than certain degree. Said pushing-down members (91/93) may also be engaged with the top member (99) by means of screws and, in that case, said members (91/93) are in such a constitution that they can be pushed against the side of the container (81) by rotating them.

Anyway, the constitution is to be in such a manner that, until there is a need of taking out the medium and the disinfectant in use of the microorganism-detecting apparatus according to the present invention, the bag-shaped members such as ampules are sustained freely from being broken and releasing its content but, once necessary, each of the contents can be freely or optionally released therefrom.

FIG. 16 illustrates a fundamental arrangement required in a representative specific embodiment of the microorganism-detecting apparatus according to the present invention, particularly the fundamental arrangement for disinfecting and/or sterilizing the cultured medium, etc. after incubation.

In FIG. 16, a cylindrical cover (102) is mainly composed of (i) a cover-constituting member (112) which constitutes an almost lower half of the cover (102) and (ii) another cover-constituting member (111) which constitutes an almost upper half of the cover (102). At least one of the cover-constituting members (112 and 111) is made in such a manner that it can rotate against another by applying a force thereto along the circumference of the cover (102). In the cover (102), both an ampule (104) containing a medium and another ampule (108) containing a disinfectant are received and held. The inner side of the cover-constituting member (112) is equipped with a protrusion (113) which contacts the ampules (104 and 108). The cover-constituting member (111) is equipped with holes (124 and 128) (the symbols are omitted in the drawing) which can receive the respective ampules (104 and 108). A cover (102) is constituted in such a manner that the cover-constituting member (111) is located on the cover-constituting member (112) (i.e., at the opposite side of the container (101)) and an ampule (104) is placed in a hole (124) in the cover-constituting member (111) while another ampule (108) is placed in another hole (128). In the cover-constituting member (112), the ampules (104 and 108) placed as such are located so as to encounter to the protrusion (112) as in (a) of FIG. 16.

When, for example, the upper cover-constituting member (111) is rotated to an extent of 90 degrees to an anticlockwise direction, a tension would occur at the contacting part (115) of the ampule with the protrusion (113) whereupon the ampule (104) is broken and the medium contained therein is flown out. The medium coming out as such comes into a container (101) through one or more perforated holes (not shown) at the engaging portion of the cover (102) with the

container (101) by gravity and contacts with the microorganism-collecting end (106b). When the above cover-constituting member (111) is further rotated to an anticlockwise direction (totally rotated to an extent of 180 degrees), a tension would occur at the contacting part of the ampule (108) (not shown) with a protrusion (113) whereby the ampule (108) is broken and the disinfectant contained therein is flown out. The disinfectant discharged as such comes into a container (101) by gravity through the perforated hole (not shown) at the engaging portion (joint) of the cover (102) with the container (101) and disinfects/sterilizes the apparatus including incubated microorganism. Alternatively, when the above cover-constituting member (111) is rotated to a clockwise direction instead of rotating it to an extent of 180 degrees to an anticlockwise direction as mentioned above, a tension would occur at a contacting part (116) of the ampule (108) with the protrusion (113) where by the ampule (108) is broken and the disinfectant contained therein is flown out.

The microorganism-detecting apparatus of the present invention can be made (i) in such a structure that a mechanism for breaking the bag-shaped member such as ampule does not work by being equipped with a pawl or the like so that the bag-shaped member such as ampule is sustained freely from breakage and the content in the ampule is not released until there is a need of releasing the medium or the disinfectant or (ii) in such a structure that an arrangement is made wherein, unless a certain force is applied, rotation or pushing is not possible so that the mechanism for breaking the bag-shaped member such as ampule does not work. The microorganism-detecting apparatus of the present invention may also be in such a manner that a protector such as a cap is applied to the mechanism for breaking the bag-shaped member so that the content therein is not released by breakage of the bag-shaped member such as ampule until there is a need of releasing the medium or the disinfectant.

FIGS. 17 to 33 illustrate other representative specific examples of the microorganism-detecting apparatus of the present invention for their fundamental constitution, particularly the fundamental constitution as a means for disinfecting and/or sterilizing the cultured medium, etc. after incubation.

FIG. 17 mainly shows the external appearance and shape of said microorganism-detecting apparatus while its main cross section is shown in FIG. 18.

FIGS. 19 to 33 show each of the materials constituting said microorganism-detecting apparatus in some more detail. Incidentally, in those drawings, a microorganism-collecting part or the like is sometimes omitted for better understanding.

A main appearance of a cap member (561) is shown in FIG. 19 while the cross sectional shape of said cap member (561) is mostly shown in FIG. 20. FIG. 21 is mostly a cross sectionally perspective view of the lower area seen from the section I-I' of said cap member. An appearance of the cover-constituting member (247) constituting said microorganism-detecting apparatus is mostly shown in FIG. 22 while the cross sectional view of said cover-constituting member (247) is mostly shown in FIG. 23. FIG. 24 mostly shows the appearance of said cover-constituting member (247) from the direction of II while FIG. 25 mostly shows the appearance of said cover-constituting member (247) from the direction of III. FIG. 26 mostly shows the appearance of the cover-constituting member (243) constituting said microorganism-detecting apparatus, FIG. 27 mostly shows the cross sectional view of said cover-constituting member (243), FIG. 28 mostly shows the appearance of said cover-constituting member (243) from the direction IV and FIG. 29 mostly shows the appearance of said cover-constituting member (243) from the direction V, respectively. The appearance of the container (241) which constitutes said micro-organism-detecting apparatus is mostly shown in FIG. 30, and the cross sectional view of said container (241) is mostly shown in FIG. 31. FIG. 32 mostly shows the appearance of said container (241) from the direction of VI and FIG. 33 mostly shows the cross sectional view of said container (241) at the G-G' section in said microorganism-detecting apparatus equipped with the microorganism-collecting end (246b).

In FIGS. 17 and 18, the cover (242) is mainly composed of (a) a cover-constituting member (243) which is capable of receiving and holding an ampule (244) containing a medium; (b) a cover-constituting member (247) which is capable of receiving and holding an ampule (248) containing a disinfectant; and (c) a cap member (249, 561). A protrusion (252) is installed (formed) at the bottom of the cover-constituting member (243), i.e. at the connecting side (259) with the cylindrical container (241). The protrusion (252) may be placed on the entire circumference of the cover-constituting member (243) whereby said cover-constituting member (243) becomes thick at the connecting part (259) side with the container (241). Alternatively, said protrusion may be placed (formed) in such a manner that the protrusions are located in a projected manner at some places on the inner side of the cover-constituting member (243), e.g. those in a form of a crosspiece (sash bar). In an example shown in the drawing, it is noted that said protrusion is installed (formed) having a wedge-shaped cross section at four sites as given in FIG. 28. Such a shape of said protrusion (252) is effective in giving a big breaking force at the contacting point A with the ampule (244). Other examples of the shape whereby such a function and merit can be expected may be suitably selected by anyone skilled in the art from those which have been widely known in the areas of machines and instruments, particularly in the area of containers. In a preferred embodiment, a protrusion (297) is formed at the top side of the cover-constituting member (243), i.e. at the cap member (249) side terminal area, so as to engage with a engaging recess part (299) formed on the cover-constituting member (247) and, when the head part (245) of the cover-constituting member (247) is pushed to the direction of the container (241), the bottom (235) of the cover-constituting member (247) can be pushed against the side of the container (241).



In an example shown in this drawing, said protrusion (297) is formed at four places on the inner wall of a cylindrical material (243) as same as the protrusion (252) which is shown in FIG. 28 and, corresponding to that, said connection recess part (299) is formed at four places on the outer wall of the member (247) at the side of the container (241). There is no particular limitation for their structures in connection with such protrusions (297) and connection recess parts (299) as long as they can engage each other for setting the cover-constituting member (247) at the inner side of the cover-constituting member (243) and the cover-constituting member (247) can slide to the side of the container (241) preferably at the breaking stage of the ampule (244). The shape and structure can be suitably selected from those which are widely known in the areas of machines and instruments, particularly in the area of containers, by anyone skilled in the art provided that the function and the merit as mentioned above can be expected. At the bottom (235) of the cover-constituting member (247), there are one or more through holes (perforated holes) (cf. FIG. 25) so that, when the ampule (248) is broken, the disinfectant contained therein can flow down into the container (241) as mentioned below. At the bottom side of the cover-constituting member (247), i.e. at the inner ampule (244) side, there is a protrusion (253) by the same manner as in the case of the cover-constituting member (243). Like the above-mentioned protrusion (252), the protrusion (253) may be made on the entire circumference of the cover-constituting member (247) whereby the cover-constituting member (247) becomes thick at the bottom of the ampule (244) side thereof. Alternatively, said protrusion (253) may be made in such a manner that the projected parts are partially available at several places on the inner side of the cover-constituting member (247) (such as a protrusion in a form of crosspieces). In an example shown in this drawing, it is noted that, as shown in FIG. 24, said protrusion (253) is installed in such a manner that it has a wedge-like cross section at four sites. In a preferred embodiment, thread cutting is made at the top side of the cover-constituting member (247), i.e. on the end (245) at the cap member (249) side, so as to gear with the screw (257) made on the cap member (249) and, when the head of the cap member (249) is rotated, the bottom (256) of the cap member (249) can be pushed against the side of the ampule (248).

As well noted by referring to FIG. 18 for example, when the head (245) of the cover-constituting member (247) is first pushed down to the direction of the container (241) and then the cover-constituting member (247) is pushed into the cover-constituting member (243), the bottom (235) of the cover-constituting member (247) pushes down the ampule (244). The pushed-down ampule (244) is then supported by a slope of the protrusion (252) made on the cover-constituting member (243). As such, a tension would occur at the contacting point (A) of the ampule (244) with the slope of the protrusion (252) as the cover-constituting member (247) is pushed down to the side of the container (241) whereby the lower part of the ampule (244) is broken and the content therein is allowed to release. The content released from the ampule (244) flows into the container (241) by gravity passing the through hole (perforated hole) (cf. FIG. 29) made in the first partition member (296) and contacts with the microorganism-collecting end (246b). (Incidentally, in FIG. 28, the ampule (244) is omitted and, further, in FIG. 29, the microorganism-collecting part which is to be made at the central site is omitted.) In an example shown in this drawing, there are four through holes in a fan-like shape as will be noted by referring to FIGS. 28 and 29.

During the disinfecting stage after incubation of the microorganism, when the top (561) of the cap member (249) is rotated and the bottom (256) of the cap member (249) is pushed against the side of the ampule (248), the bottom (256) of the cap member (249) pushes down the ampule (248). The pushed-down ampule (248) is then supported by the slope of the protrusion (253) made on the inner side of the cover-constituting member (247). As the cap member (249) is rotated and pushed against the side of the container (241), a tension would occur at the contacting point (B) of the ampule (248) with the slope of the protrusion (253) whereby the lower part of the ampule (248) is broken and the content therein is allowed to release. The content coming out from the ampule (248) is flown into the container (241) by gravity passing through the perforated holes (cf. FIG. 25) made at the bottom (235) of the cover-constituting member (247) and contacts with the incubated microorganism. (Incidentally, in FIG. 24, the ampule (248) is omitted.) In an example shown in this drawing, there are four perforated holes (through holes) in a sectoral form as will be noted by referring to FIGS. 24 and 25. It is preferred in view of convenient use that the ampule is made of glass, hard plastics, etc. so that it can be easily broken upon application of mechanical force (particularly upon application of a mechanical force partially).

In the microorganism-detecting apparatus shown in said drawings, the ampule containing the medium can be broken by mere sliding and pushing of the cover-constituting member and the cap member (both are screwed and unitedly movable) to supply the medium for incubation to the container while another ampule containing a disinfectant/sterilizer can be broken to release the disinfectant/sterilizer contained therein into a container for incubation only when the cap member screw-fitted with the cover-constituting member accommodating said disinfectant/sterilizer ampule is rotated and pushed thereagainst. Therefore, said microorganism-detecting apparatus is excellent in terms of actual use.

In the microorganism-detecting apparatus of the present invention, it is also possible that a lock function is installed so that initiation of said sliding operation does not take place unless a certain force is applied thereto. It is further possible that initiation of the rotary movement of the cap member does not take place unless a certain force is applied thereto. With regard to a structure giving such a lock function, those which are known in the area of containers may be selected and used. There is no particular limitation for the size of said microorganism-detecting apparatus but, in terms

of portability, the total size will be, for example, about 5 to 40 cm in length (preferably, about 10 to 30 cm or, more preferably, about 15 to 25 cm) and about 3 to 50 mm in diameter (preferably, about 6 to 30 mm or, more preferably, about 8 to 25 mm). The size of said microorganism-detecting apparatus may be other than those given hereinabove and freely selected and designed by anyone skilled in the art depending upon the object and operability and also by taking the quality, etc. of the stuff constituting said apparatus into consideration.

FIGS. 34 to 51 illustrate other representative specific examples of the microorganism-detecting apparatus of the present invention for their fundamental constitution, particularly the fundamental constitution as a means for disinfecting and/or sterilizing the cultured medium, etc. after incubation.

FIG. 34 illustrates the assembling of the parts which constitute the microorganism-detecting apparatus according to the present invention. FIG. 34 shows a cap member (761), an ampule (748) wherein a disinfectant is contained, a cover-constituting member (747) capable of receiving and holding the ampule (748), an ampule (744) containing a medium, a cover-constituting member (743) capable of receiving and storing the ampule (744), a microorganism-collecting member (746) (comprising a rod-shaped element (746a) and a microorganism-collecting end (746b)) and a container (741). The assembling concept as shown in FIG. 34 is applicable to the microorganism-detecting apparatus of the present invention shown in FIGS. 17 to 33 as well.

FIG. 35 mostly shows the cross-sectional shape of said microorganism-detecting apparatus. FIG. 36 mostly shows the appearance of the cover-constituting member (747) constituting said microorganism-detecting apparatus which is similar to that as shown in FIG. 22. FIG. 37 mostly shows the cross-sectional view of said cover-constituting member (747) along the line M-M'; FIG. 38 mostly shows the appearance of said cover-constituting member (747) observed from the direction of VII; and FIG. 39 mostly shows the appearance of said cover-constituting member (747) observed from the direction of VIII (In FIGS. 38 and 39, a bridge part at the end of said cover-constituting member (747) and at the container side, connecting to the protrusion (753), is observed wherein the thing which is shown as if obliquely crossing to said bridge is a "flash" which is incidentally formed upon molding of the plastic resin and, preferably, such a flash is to be detached in the actual product.)

FIG. 40 mostly shows the cross-sectional shape of the cover-constituting member (747) which constitutes said microorganism-detecting apparatus similar to that shown in FIG. 23 wherein (778) is a small convex ridge formed on the inner wall of the cover-constituting member (747) while (753) is a protrusion for breaking the ampule (748). Unlike the (253) shown in FIG. 23 (and also in FIG. 24), this (753) is formed only at one place (cf. FIG. 38) and, in addition, the shape is different (cf. FIG. 40). In the microorganism-detecting apparatus, such an ampule-breaking function and a structure for enabling said function are also characteristic features of the present invention. Accordingly, an apparatus and materials (members) having such a function constitute a part of the present invention.

As will be noted from FIG. 37, there is a concave (□) and shallow groove (777) in said cover-constituting member (747). Said groove (777) is formed in such a manner that, as will be illustrated later, it can be engaged with a small convex ridge (781, 782, 783, etc.) formed on the inner wall of the cover-constituting member (743) which constitutes said microorganism-detecting apparatus whose cross-sectional shape is mostly shown by FIG. 44. Although, in an example as shown in said drawing, length of all of four grooves (777) is same, numbers and length of the groove may be suitably changed upon necessity. In FIG. 40, no perforated hole is illustrated through which a disinfectant (liquid) contained in the ampule (748) flows down by gravity when the ampule (748) is broken, but said perforated hole (through hole) can be easily understood by referring to FIGS. 38 and 39.

In FIGS. 35 and 40, it is shown that the inner diameter of the about one-third portion of the cover-constituting member (747) at the side of the container (741) is either broader than or the same as the inner diameter at the side of its cap member (761) although it is also preferred that the inner diameter is narrower than that at the side of its cap member (761) so that the movement of the receiving ampule (748) is restricted.

FIG. 41 mostly shows the appearance of the cover-constituting member (743) which constitutes said microorganism-detecting apparatus which is the same as that as shown in FIG. 26. FIG. 42 mostly shows the appearance of the cover-constituting member (743) observed from the direction of IX; and FIG. 43 mostly shows the appearance of said cover-constituting member (743) from the direction of X. By referring to FIGS. 35, 42 and 43, the presence of the through holes (perforated holes) through which the medium (liquid) contained in the ampule (744) flows down by gravity can be easily understood. In this example, there are three perforated holes (through holes) in a shape of a fan. When the ampule (748) is broken, the disinfectant (liquid) contained in said ampule (748) flows down by gravity through said perforated holes and comes into the container (741).

There are small convex (△) ridges (781, 782, 783 and 784) on the inner wall of said cover-constituting member (743). In FIG. 44, there are two small convex ridges (781 and 783) in the plane N-N' (cf. FIG. 45 which illustrates a cross section along the plane N-N') but, in the P-P' plane, there are four (781, 782, 783 and 784) (cf. FIG. 46 which illustrates a cross section along the plane P-P'). FIG. 47 shows a cross section along the plane Q-Q'. The small convex ridge (781) ranges consecutively to (785) and further extends to (752) while, among others such as (782), it ranges to (786) but has no protrusion such as (752). Unlike (252) shown in FIG. 27, there is only one (752) in the present microorganism-detecting apparatus and its shape is different as well. In the microorganism-detecting apparatus of the present invention, such



an ampule-breaking function and a structure which makes it possible are also the characteristic features of the present invention and apparatus and materials (members) having such functions also constitute a part of the present invention.

FIG. 48 shows the oblique outer view of the cover-constituting member (743) made up of a transparent (or translucent) stuff in a partly perspective manner. In said drawing, the arrangement of small convex ridges (781, 782 and also 785 and 752 ranging therefrom) formed on the inner wall of the cover-constituting member (743) is visible and understandable. (781) extends to the cover-constituting member (747)-receiving side longer than (782). Said small convex ridges may be made in such a manner that the convex is made larger (i.e. projected to a higher extent) from the place (785) ranging therefrom for allowing the received ampule (744) to be loosely fixed. The area (790) at the outer wall of the cover-constituting member (743) is made hardly slippery by forming a finely indented surface, like a frosted glass. Such a finely indented surface, like a frosted glass, may be at either a part of or whole of the cover-constituting member (743).

The basic shape of the apparatus shown in FIGS. 34 to 51 is the same as that of the microorganism-detecting apparatus of the present invention shown in FIGS. 17 to 33 but its characteristic feature is as follows:

Until an initiation of incubation, said cover-constituting member (741) is set free by means of a locking mechanism from pushing into said cover-constituting member (743) to break the ampule (744) received in said cover-constituting member (743), while, upon the initiation of incubation, the engagement is easily unlocked by merely rotating said cover-constituting member (747) against said cover-constituting member (743) whereby said cover-constituting member (747) can be pushed into said cover-constituting member (743) so that the ampule (744) is broken and the medium can be supplied to the container (741).

FIGS. 49 to 51 illustrate the conceptual outlines of said locking mechanism and unlocking thereof. At the state prior to use of said microorganism-detecting apparatus, when the cover-constituting member (747) is inserted into the cover-constituting member (743) in such a manner that (i) a long and convex (凸) small ridge (781) formed on the inner side of the cover-constituting member (743) and (ii) a concave (凹) and shallow groove (777) formed on the inner side of the cover-constituting member (747) are not engaged each other, both said cover-constituting members are fixed due to a big frictional engaging force between the convex small ridge (781) and the outer surface of said member (747). As a result, each member stays nearly at the position as shown by FIG. 49. In other words, the fixation is in such a manner that said member (747) cannot be pushed into said member (743) (i.e. against the side of the container) beyond said stopping position.

When, upon breaking the ampule (744), for example, the cover-constituting member (747) is rotated to the direction as shown by an arrow in FIG. 49, (i) the convex small ridge (781) and (ii) the concave and shallow groove (777) are engaged each other as shown in FIG. 50, so that the frictional engaging force generated between the convex small ridge (781) and the outer surface of the cover-constituting member (747) disappears whereupon the cover-constituting member (747) can now be pushed to the direction further as shown by an arrow in FIG. 50. Then, when the cover-constituting member (747) is inserted as shown in FIG. 51, the bottom of the cover-constituting member (747) pushes the head of the ampule (744) whereupon the ampule is pushed to the protrusion (752) followed by breakage. In this example, (i) the convex ridges (four in total) formed on the inner side of the cover-constituting member (743) and (ii) the concave shallow grooves (four in total) (recessing parts) formed at the outer side of the cover-constituting member (747) are engaged each other at the end. Simultaneously, the engaging surface of the cover-constituting member (743) and that of the cover-constituting member (747) are tightly engaged each other at the side of the cap member (761). Thus, the inner wall of the cover-constituting member (743) and the outer wall of the cover-constituting member (747) are tightly contacted each other, thereby achieving a tight sealing to some extent. In the microorganism-detecting apparatus, such a locking mechanism and a structure for making it possible or available are a part of the characteristic features of the present invention and the apparatus having such a mechanism (or function) and materials (members) thereof constitute a part of the present invention.

The microorganism-detecting apparatus of the present invention as shown in FIGS. 34 to 51 are the same as those shown in FIGS. 17 to 33 in terms of basic shape, size, etc. and, accordingly, details of other materials will be and is to be understood similarly.

FIG. 52 partly shows a partial cross sectional view of the connecting part (776a) of the cover-constituting member (743) with the container (741). It is understood that an end of the microorganism-collecting member (746) can be inserted into and fixed with the inlet (789) for the microorganism-collecting part (member). At the basement of said inlet (789), there is one or more perforating holes for communicating a medium and a disinfectant. As will be noted by referring to FIG. 52, a protrusion (789a) is formed in a manner of surrounding the inlet (789). When an end of the container (741) is screwed into a connecting part (776a) of the cover-constituting member (743), said protrusion (789a) assists a close engagement (connection) of said cover-constituting member (743) with the container (741). In other words, when a plastic resin such as polypropylene is used as a constituting stuff for the engaging portions connecting between said cover-constituting member (743) and the container (741), it is possible to tightly seal it through utilizing the elasticity of the stuff for the members. In the microorganism-detecting apparatus according to the present invention, the structure of such an engaging part (776a) of the cover-constituting member (743) with the container (741) is also one of the char-

acteristic features of the present invention and the apparatus having such a function and materials thereof also constitute a part of the present invention.

FIGS. 53 to 69 illustrate other representative specific embodiments of the microorganism-detecting apparatus according to the present invention for their fundamental constitution, particularly the fundamental arrangements suitable for disinfecting and/or sterilizing the cultured medium, etc. after incubation.

In these drawings, the microorganism-detecting apparatus according to the present invention is shown which has a mechanism for allowing us to keep and carry it securely and which is designed in a manner that we can avoid the breakage of (i) an ampule containing a disinfectant and (ii) an ampule containing a medium.

FIGS. 53, 55, 57, 59, 61, and 63 show a perspective view of the microorganism-detecting apparatus according to the present invention, respectively. FIG. 54 illustrates the assembling of the parts 1245 (and 1561) and 1243 for the microorganism-detecting apparatus of FIG. 53. FIG. 56 illustrates the assembling of the parts 1245 (and 1561) and 1243 for the microorganism-detecting apparatus of FIG. 55. FIG. 58 illustrates the assembling of the parts 1561 and 1245 for the microorganism-detecting apparatus of FIG. 57. FIG. 60 illustrates the assembling of the parts 1561 and 1245 for the microorganism-detecting apparatus of FIG. 59. FIG. 62 illustrates the assembling of the parts 1561, 1245 and 1243 for the microorganism-detecting apparatus of FIG. 61. FIG. 64 illustrates the assembling of the parts 1561, 1245 and 1243 for the microorganism-detecting apparatus of FIG. 63.

In FIGS. 53 and 57, a guide groove (guide space) (1004, 1007) is formed at two sites while the guide groove (guide space) is formed at one site in FIGS. 55 and 59. In FIG. 61, the member (1243) has two guide grooves (guide spaces) (1004); the member (1245) has two stoppers (1005) and two guide grooves (guide spaces) (1007); and the cap member (1561) has two stoppers (1008)). In FIG. 63, the member (1243) has one guide groove (guide space) (1004); the member (1245) has one stopper (1005) and one guide groove (guide space) (1007); and the cap member (1561) has one stopper (1008). One or optionally more guide grooves (guide spaces) can be formed in each member. Although, in the drawings, the guide portion (1004) is formed through the side wall of the member (1243), it is possible to form a shallow guide groove by reducing the inner side wall of the member (1243) so as to make it partially thin (by cutting the inner side wall thereof) without a through hole. In the drawings, the guide (1004) is formed in the side wall of the member (1243) and a stopper (1005) is formed on the side wall of the member (1245) at the engaging side with the member (1243). Alternatively, the guide (1004) may be formed in the side wall of the member (1245) and a stopper (1005) may also be formed on the side wall of the member (1243). Similarly, a guide portion (1007) and a stopper (1008) can be formed in the members. The shape of the guide groove (guide space) is not limited to but includes a L shape, those as illustrated in FIG. 70 and the like.

FIG. 65 shows an appearance of the members (1561, 1245) in another representative embodiment of the microorganism-detecting apparatus according to the present invention. FIG. 66 shows an appearance of the members (1561, 1245) in another representative embodiment of the microorganism-detecting apparatus according to the present invention. FIG. 67 shows the member 1243 capable of engaging with the members (1245 (and 1561)) illustrated in FIG. 65. FIG. 68 is a schematic diagram for explaining the relationship between the guide portion (1004) and the stopper (1005). FIG. 69 is an enlarged partial view in connection with the relationship between the guide portion (1004) and the stopper (1005).

Prior to use of the microorganism-detecting apparatus according to the present invention, the member (1245) is engaged with the member (1243) (FIG. 54) and the stopper (1005) is positioned at the upper side of the guide (1004) (FIG. 53). Referring to FIG. 68, the stopper (1005) is located at the site B in FIG. 68-1.

Upon breaking the ampule (upon use thereof), the stopper (1005) is moved beyond a pawl portion (neck) (A) (FIG. 68-2 and FIG. 69) by turning the member (1245) whereby the member (1245) can be pushed into the member (1243). Thus, the stopper (1005) can be moved to the position (D) (In FIG. 53, it corresponds to the stopper (1005')). If necessary, a pawl portion (neck) (C) may be formed in order to fix the stopper (1005) (FIG. 68-3). In FIGS. 55 to 64, each apparatus has the same mechanism and action.

FIG. 65 shows an embodiment of the microorganism-detecting apparatus according to the present invention wherein a means for securing the adhesion (sealing) between the member (1243) and the member (1245) is set at the surface region (1351) on the outer wall of the member (1245). The adhesion (sealing) may be achieved by various techniques including frosting of surface, coating with rubber, etc. and the useful technique can be selected from those known to a person skilled in the art such as the field of container. Although FIG. 65 also shows the possibility that a means for securing the adhesion between the member (1243) and the member (1245) will be made at the surface region (1350) on the outer wall of the member (1245), said means is generally disposed at the surface region (1351) when the stopper (1005) -guide (1004) system is formed.

FIG. 67 shows an embodiment of the microorganism detecting apparatus according to the present invention wherein a means for securing the adhesion between the members (deeply shadowed area) is installed on the inner wall of the member (1243) which corresponds to the surface region (1351) formed on the member (1245). Similarly to FIG. 65, FIG. 67 also show the possibility that a means for securing the adhesion will be made on the slantingly lined area. In FIG. 67, a shallow groove (1360) is formed so as to facilitate an insertion of the stopper (1005) into the guide portion

(1004).

In FIG. 66, the stopper (1352) has a structure capable of being flexibly pressed down against the outer wall side of the member (1245) upon insertion of the member (1245) into the member (1243). The stopper (1352) is movable inwardly and outwardly by reflecting a force. In addition, the mechanism required in keeping and carrying the apparatus without the breakage of (a) the ample for a medium and (b) the ample for a disinfectant may include an engagement of a convex portion formed on one member with a concave portion formed on another member for (i) the members (1245) and (1243) and for (ii) the members (1561) and (1245), a combination of a stopper removable from the detecting apparatus therewith. Said mechanism may comprise a mark attached thereto, which indicates a position for fixing each member.

In accordance with the present invention, it has been found that it is possible to measure how many viable microorganism cells are present prior to an initiation of incubation, substantially in a closed system, such as the microorganism-detecting apparatus of the present invention. Utilization of the microorganism-detecting apparatus of the present invention enables us to easily, securely and portably estimate the number of viable microorganism cells present in foods, cooking tools and the like. Accordingly, it is possible to estimate a degree of pollution quickly for various samples including cooking utensils, e.g. kitchen knife and chopping board, foods, and beverages or surroundings, by using the microorganism-detecting apparatus of the present invention. Through the use of the apparatus of the present invention, we can easily, conveniently, securely and safely judge what kind or species of microorganism it is and/or determine how much amount of microorganisms are present.

The measurement of viable cells can be carried out by culturing the cell in a liquid medium contained in the apparatus of the present invention followed by measuring absorbance, turbidity, etc. for the medium without taking out of the apparatus. The measurement may be carried out by a measuring instrument or machine, by visual observation including a comparison with a symbol mark and the like, or by a combination thereof. The visual observation may include a technique employing labels such as characters, lines, pictures and dots wherein a recognition degree is estimated. The label for recognition may be attached to or marked (painted) on a swab (microorganism-collecting member), an inner wall of the container receiving the medium for culturing cells, or an outer wall thereof. The label for recognition may be suitably selected in view of its shape, color, depth and lightness. For the quantitative measurement, it is preferred to avoid a pigment in a medium for culturing microorganisms. In the present measurement, it is possible to estimate the number of cells present at a starting point.

Incidentally, in FIG. 1 and in FIGS. 3 to 70, illustrations are made for concentrating to the characteristic points of the microorganism-detecting apparatus according to the present invention. It is to be understood that some of the details may be omitted there. Consequently, it is to be understood that the apparatus having the characteristic features of the apparatus according to the present invention shown in this specification, drawings, and the attached claims including those wherein various modifications are made and each of the constituting parts or members is connected each other in more detailed manner are also included in the coverage of the present invention.

#### MERIT OF THE INVENTION

As mentioned hereinabove, the present invention provides a selective microorganism-detecting apparatus where certain microorganisms (particularly pathogenic ones and, more particularly, those for food poisoning) can be easily, securely and simply detected and/or identified. Said apparatus can be expected to be stored for long period and is in such a shape (arrangement) that it can be immediately used at any time when necessary and that it can be easily and conveniently carried. Thus, the present invention provides a selective microorganism-detecting apparatus which is especially suitable for private and domestic use where toxic microorganisms such as those for food poisoning can be easily, securely and simply detected/identified and, even after use, is able to be disposed safely, surely and easily.

The microorganism-detecting apparatus of the present invention can be very easily used privately and domestically and, therefore, it is now possible to detect the microorganism and also to establish a preventing measure against food poisoning by a self-active basis being not participated by professionals such as those in public health centers. After incubation of the microorganism, the microorganism-detecting apparatus of the present invention is not made into an open system, for instance, by opening a cover. Even if pathogenic microorganism is contained therein, therefore, it is easy to change the used apparatus to a disposable form and then to discard it. Moreover, there is no need that the apparatus used for incubation is opened and disinfected by adding a disinfectant to the microorganism-containing culture liquid with a tool such as a pipette. Accordingly, troublesome operations can be eliminated and there is no danger. No professional technique, skillfulness and apparatus is necessary to operate the apparatus of the present invention for detecting microorganisms. Further, in the microorganism-detecting apparatus of the present invention, it is unnecessary to prepare a disinfectant solution at all times separately from the container for incubation. Therefore, the apparatus is quite good in terms of portability and easy handling.

EXAMPLES

Described below are examples of the present invention which are provided only for illustrative purposes, and not to limit the scope of the present invention. In light of the present disclosure, it should be understood that numerous embodiments within the scope of the claims will be apparent to those of ordinary skill in the art.

Example 1

(Apparatus for Detection of Staphylococcus aureus and Preparation Thereof)

By referring to FIG. 5, the following materials were used to construct the microorganism-detecting apparatus having the following size:

Container (1): a hollow cylinder having a bottom (inner diameter: 8.5 mm; height: 150 mm) made of polyethylene (thickness: 0.6 mm)

Cover (2): a hollow cylinder having a bottom (inner diameter: 10 mm; length: 53 mm) made of polyethylene (thickness: 0.6 mm)

First bag-shaped member (4): a hollow cylinder having a bottom (inner diameter: 7.5 mm; length: 40 mm) made of glass (thickness: 0.5 mm)

Partition member (5): a disk-shaped body with a diameter of 6 mm made of polyethylene (thickness: 1 mm)

Rod-shaped element (6a): made of polyethylene; diameter: 2 mm; length: 135 mm

Microorganism-collecting end (6b): an end of "swab", made of absorbent cotton (diameter: 5 mm; length: 15 mm)

Disk member (7): filter paper (thickness: 1 mm; diameter: 6 mm)

The antibiotic substances as shown in Table 1 were impregnated in a disk member (7), located between the bottom of the container (1) and the microorganism-collecting end (6b) in such amounts that concentrations of 10 µg/ml of aztreonam, 8 µg/ml of polymyxin B and 5 µg/ml of fluconazole were obtained upon unification with the medium.

Medium (3): a medium having the composition as shown in Table 1 (except the antibiotics).

Table 1

A) Screening Medium for Detection of Staphylococcus aureus  
(Modified Mannitol-Salt Medium)

Myosate Peptone	2.5 g
Polypeptone Peptone	10.0 g
Yeast Extract	2.5 g
D-Mannitol	10.0 g
Lithium Chloride	5.0 g
Sodium Chloride	40.0 g
Phenol Red	25 mg
Distilled Water	1000 ml

pH 7.5

Sterilized by autoclaving at 115 °C for 15 minutes.

Secondary Test: Disk for detection of phosphatase

Disk:	Aztreonam	1 to 15 $\mu$ g/ml
	Polymyxin B	1 to 15 $\mu$ g/ml
	Fluconazole	1 to 10 $\mu$ g/ml

After preparing a detecting apparatus for Staphylococcus aureus having the above composition, it was sterilized by irradiating with gamma-ray at 1 to 30 °C for one minute. The apparatus after the sterilization was stored in a bag made of polyethylene film (thickness: 100  $\mu$ m) laminated with (vaporized) aluminum for shielding the light until immediately before use.

Example 2

(Detecting Apparatus for Vibrio parahaemolyticus and Preparation Thereof)

A detecting apparatus for detecting Vibrio parahaemolyticus was prepared, sterilized and stored by the same manner as in Example 1 with an exception that composition for the medium (3) and antibiotics were changed as shown below (Table 2) by referring to FIG. 5. At that time, the antibiotic substances as shown in Table 2 were impregnated in a disk material (7) in such amounts that concentrations of 10  $\mu$ g/ml of polymyxin B, 5  $\mu$ g/ml of fluconazole and 5  $\mu$ g/ml of potassium tellurite were obtained upon unification with the medium.

Table 2

B) Screening Medium for Detection of Vibrio parahaemolyticus  
(Modified Salt-Polymyxin Medium)

Polypeptone	Peptone	10.0 g
Yeast Extract		5.0 g
Sodium Chloride		20.0 g
Saccharose		15 g
Sodium dodecyl sulfate		1.0 ml
Bromthymol Blue		40.0 mg
Cresol Red		40.0 mg
Distilled Water		1000 ml
pH 7.2		

Sterilized by autoclaving at 115 °C for 15 minutes.

Secondary Test: Disk for detection of cytochrome oxidase

Disk:	Polymyxin B	1 to 15 $\mu$ g/ml
	Fluconazole	1 to 10 $\mu$ g/ml
	Potassium tellurite	1 to 10 $\mu$ g/ml

Example 3

(Detecting Apparatus for Salmonella and Preparation Thereof)

A detecting apparatus for detecting Salmonella was prepared, sterilized and stored by the same manner as in Example 1 with an exception that composition for the medium (3) and antibiotics were changed as shown below (Table 3) by referring to FIG. 5. At that time, the antibiotic substances as shown in Table 3 were impregnated in a disk member (7) in such amounts that concentration of 5  $\mu$ g/ml of fluconazole was obtained upon unification with the medium.

Table 3

C) Screening Medium for Detection of Salmonella  
(Modified Xylose-Lysine Medium)

10	Yeast Extract	5.0 g
	Sodium Chloride	5.0 g
	Saccharose	5.0 g
15	Lactose	5.0 g
	Xylose	4.0 g
	Lysine	10.0 g
	Sodium dodecyl sulfate	1.0 ml
20	0.2% Bromocresol Purple	12.0 ml
	Ammonium Iron Citrate	0.5 g
	Sodium Thiosulfate Pentahydrate	0.2 g
25	Distilled Water	1000 ml
	pH 6.8	
	Sterilized by autoclaving at 115 °C for 15 minutes.	

30 Disk: Fluconazole 1 to 10  $\mu$  g/ml

35 Example 4

(Confirmation of Selectivity of a Detecting Medium for Staphylococcus aureus)

40 Selectivity of the medium for detecting Staphylococcus aureus prepared in Example 1 was confirmed to be as follows:

45 Twenty-two kinds of microorganisms as shown in the following Table 4 were dispersed in 1.5 ml of the medium for detecting Staphylococcus aureus prepared in Example 1 to make the concentration about  $10^6$  cells/ml at the initiation of the incubation, cultured in air at 37 ° C for 24, 48 or 72 hours and, out of the color change (if any) of Phenol Red (a pH indicator contained in the above medium), a judgment was made whether the growth of the microorganism was noted. When the color tone of the medium after the incubation turned yellow or red, the conclusion was done as "positive (+)" (growth of the microorganism was noted) or "negative (-)" (growth of the microorganism was not noted), respectively.



TABLE 4

A) Modified Mannitol Salt Base Medium (Gram-Positive Cocci and Gram-Negative Cocci)

Gram Positive/Antibiotics	S. aureus 208P	S. aureus TEN	S. aureus 003	S. agal- actiae	S. pyo- genes	S. epider- midis 2	S. epider- midis 5	S. sapro- phyticus	S. haemo- lyticus	E. faeca- lis 18	E. faeca- lis 7862
Aztreonam + 10 µg/ml	+	+	+	-	-	-	-	+	+	-	-
Polymyxin B + 8 µg/ml	+	+	+	-	-	-	-	+	+	-	-
Fluconazole + 5 µg/ml	+	+	+	-	-	-	-	+	+	-	+
Gram Negative/Antibiotics	Salmo- nella	Serratia	Enterobacter	Shigella	Escherichia	Klebsiella	Proteus	Proteus	Proteus	Pseudo- monas 1	Pseudo- monas 2
Aztreonam + 10 µg/ml	-	-	-	-	-	-	-	-	-	-	-
Polymyxin B + 8 µg/ml	-	-	-	-	-	-	-	-	-	-	-
Fluconazole + 5 µg/ml	-	-	-	-	-	-	-	-	-	-	-

Positive (+): Color of the medium turned yellow; Negative (-): Color of the medium turned red

The result is as shown in Table 4. It is clear from the result of Table 4 that, when the medium for detecting Staphylococcus aureus of the present invention was used, proliferation of Gram-negative microorganism and Eumycetes was

effectively inhibited by antibiotic and antifungal substances adsorbed with said medium (3) and said disk (7).

Among the microorganisms tested hereinabove, S. epidermidis did not decompose mannitol contained in the medium (3) and, therefore, there was no change in the color tone of the medium.

According to the experiments conducted by the present inventors, it was found that there were some microorganisms other than Staphylococcus aureus which were "positive" in this medium and that they were coagulase-negative Staphylococcus (CNS), E. faecalis, E. faecium, etc. However, those microorganisms were able to be discriminated from Staphylococcus aureus by a phosphatase test or by a coagulase test (cf. "Directions for Hygienic Tests: Staphylococcus").

In a phosphatase test, incubation was conducted by the above-mentioned medium using a disk for detection of phosphatase (filter paper adsorbed with 4-methylumbelliferyl phosphoric acid) and, after that, fluorescence of 4-methylumbelliferone was detected by irradiating with ultraviolet ray (366 nm) whereby its presence was able to be confirmed.

#### Example 5

#### (Confirmation of Selectivity of a Detecting Medium for Vibrio parahaemolyticus)

Selectivity of the medium for detecting Vibrio parahaemolyticus prepared in Example 2 was confirmed to be as follows:

Twenty-two kinds of microorganisms as shown in the following Table 5 were dispersed in 1.5 ml of the medium for detecting Vibrio parahaemolyticus prepared in Example 2 to make the concentration about  $10^6$  cells/ml at the initiation of the incubation, cultured in air at 37 ° C for 24, 48 or 72 hours and, out of the color change (if any) of Bromthymol Blue and Cresol Red (pH indicators contained in the above medium), a judgment was made whether the growth of the microorganism was noted. When the color tone of the medium after the incubation turned yellow or green, the conclusion was done as "positive (+)" (growth of the microorganism was noted) or "negative (-)" (growth of the microorganism was not noted), respectively.

TABLE 5

B) Modified Polymyxin Salt Base Medium (Gram-Positive Cocci and Gram-Negative Cocci)

Gram Positive/Antibiotics	S. aureus 209P	S. aureus TEN	S. aureus 003	S. agalactiae	S. pyogenes	S. epidermidis 2	S. epidermidis 5	S. saprophyticus	S. haemolyticus	E. faecalis 18	E. faecalis 7862
	24hr	48hr	72hr	24hr	48hr	72hr	24hr	48hr	72hr	24hr	48hr
Polymyxin B 10 µg/ml + Fluconazole 5 µg/ml + Potassium Tellurite	-	-	-	-	-	-	-	-	-	-	-
Gram Negative/Antibiotics	Salmonella	Serratia	Enterobacter	Shigella	Escherichia	Klebsiella	Proteus	Proteus 19	Proteus 20	Pseudomonas 1	Pseudomonas 2
Polymyxin B 10 µg/ml + Fluconazole 5 µg/ml + Potassium Tellurite	-	-	-	-	-	-	-	+	+	-	-
	-	-	-	-	-	-	+	+	+	-	-
	-	-	-	-	-	-	+	+	+	-	-

Positive (+): Color of the medium turned yellow; Negative (-): Color of the medium turned green

The result is as shown in Table 5. It is clear from the result of Table 5 that, when the medium for detecting *Vibrio parahaemolyticus* of the present invention was used, growth of Gram-positive microorganisms was inhibited by antibi-

otic substances while that of Gram-negative microorganism and most of Eumycetes were effectively inhibited by antibiotic substances (polymyxin B, fluconazole and potassium tellurite) adsorbed with said disk (7).

According to the experiments conducted by the present inventors, it was found that there were some microorganisms other than Vibrio parahaemolyticus which were "positive" in this medium and that they were V. cholerae, V. vulnificus, Proteus spp., etc. However, those microorganisms were able to be discriminated from Vibrio parahaemolyticus by an oxidase test (a cytochrome oxidase reaction; cf. Kovacs: Nature (London), 178, 703 (1956)).

#### Example 6

##### 10 (Confirmation of Selectivity of a Detecting Medium for Salmonella)

Selectivity of the medium for detecting Salmonella prepared in Example 3 was confirmed to be as follows:

Twenty-two kinds of microorganisms as shown in the following Table 6 were dispersed in 1.5 ml of the medium for detecting Salmonella prepared in Example 3 to make the concentration about  $10^6$  cells/ml at the initiation of the incubation, cultured in air at 37 ° C for 24, 48 or 72 hours and, out of the color change (if any) of Bromcresol Purple (a pH indicator contained in the above medium), a judgment was made whether the growth of the microorganism was noted. When the color tone of the medium after the incubation turned "black" or "purple or yellow", the conclusion was done as "positive (+)" (growth of the microorganism was noted) or "negative (-)" (growth of the microorganism was not noted), respectively. Hydrogen sulfide-producing microorganisms such as Salmonella changed the color of the medium to black by the reaction of thiosulfate contained in the medium with ammonium iron citrate.

TABLE 6

C) Modified Xylose-Lysine Base Medium (Gram-Positive Cocci and Gram-Negative Cocci)

Gram Positive/Antibiotics	S. aureus 209p	S. aureus TEN	S. aureus 003	S. agal- actiae	S. pyo- genes	S. epider- midis 2	S. epider- midis 5	S. sapro- phyticus	S. haemo- lyticus	E. faeca- lis 18	E. faeca- lis 7862
Fluconazole 5 µg/ml	24hr	-	-	-	-	-	-	-	-	-	-
	48hr	-	-	-	-	-	-	-	-	-	-
	72hr	-	-	-	-	-	-	-	-	-	-
Gram Negative/Antibiotics	Salmo- nella	Serratia	Enter- obacter	Shigella	Escher- ichia	Klebsi- ella	Proteus	Proteus	Proteus	Pseudo- monas 1	Pseudo- monas 2
Fluconazole 5 µg/ml	24hr	+	-	-	-	-	-	-	-	-	-
	48hr	+	-	-	-	-	-	-	-	-	-
	72hr	+	-	-	-	-	+	-	-	-	-

Positive (+): Color of the medium turned yellow; Negative (-): Color of the medium turned purple or yellow

Salmonella .....color of the medium:

black

Escherichia coli

Citrobacter spp

Klebsiella spp

Enterobacter spp

Proteus spp

color of the medium:

yellow

Shigella spp

Serratia spp

Pseudomonas spp

color of the medium:

purple

The result is as shown in Table 6. It is clear from the result of Table 6 that, when the medium for detecting Salmonella of the present invention was used, growth of Gram-positive microorganisms was inhibited by the antibiotic sub-

stances while that of Eumycetes was effectively inhibited by the antibiotic substances (fluconazole) adsorbed with said disk (7).

According to the experiments conducted by the present inventors, it was found that there were some Gram-negative microorganisms changed the color tone of this medium from purple to yellow. Examples of such a color change were as follows:

Microorganism which changed the color to black color: Salmonella;

Microorganisms which changed the color to yellow: Escherichia coli, Citrobacter spp., Klebsiella spp., Enterobacter spp. and Proteus spp.; and

Microorganisms which changed the color to purple: Shigella spp., Serratia spp. and Pseudomonas spp.

#### Example 7

(Use of the Apparatus for Detecting Staphylococcus aureus)

A bag for the apparatus for detecting Staphylococcus aureus prepared in Example 1 was broken to take out said apparatus, then a cover (2) (and a microorganism-collecting part (6)) were taken out from the apparatus (cf. FIG. 2(a)) and the microorganism-collecting end (6b) was rubbed for several times against the surface of a cooking device (such as a chopping board) (cf. FIG. 2(b)).

After that, the above cover (2) was inserted into the apparatus again, the microorganism-collecting end (6b) was kept at a predetermined position in the container, the first bag-shaped member (4) was broken by strongly compressing from outside of the cover (2) using a compressing device ("a breaking device" in a broad clip-like shape) (not shown) and the medium (3) enclosed in said first bag-shaped member (4) was made to fall down into the container. As a result, the microorganism-collecting end (6b) in the the microorganism-collecting part (6) dipped into the medium (3) (it was possible to prevent the stuffs other than the medium (such as broken pieces of the first bag-shaped member (4)) from falling-down into the container).

Incubation was then conducted at 37 ° C for 16 to 24 hours in such a state that the microorganism-collecting end (6b) of the microorganism-collecting part (6) was dipped in a medium (3) whereupon the color tone of the medium (3) changed from red to yellow in some of the cooking device (chopping board). Thus, the presence of Staphylococcus aureus was confirmed. The cooking device where the presence of Staphylococcus aureus was confirmed as such was wiped for several times with a gauze moisturized with a 0.2 to 0.5% aqueous chlorohexidine solution ("Hibiden" (trade name); manufactured by SUMITOMO PHARMACEUTICALS CO. LTD., Japan) followed by washing for ten minutes with tap water. The cooking device (chopping board) which was treated by cleaning with invert soap and washing with water as such was subjected to a test for detecting Staphylococcus aureus as same as above whereupon the color tone of the medium (3) was unchanged but was still in yellow.

The meant that no Staphylococcus aureus was detected. Thus, it was confirmed that Staphylococcus aureus could be effectively removed by the above-mentioned disinfecting treatment.

(Disinfecting/Sterilizing Treatment in the Used Apparatus for Detecting Staphylococcus aureus)

The second bag-shaped member (8) was broken by strongly compressing its cover (2) from outside using a compressing device (a "breaking device" in a broad clip-shaped one) (not shown) whereby a disinfectant/sterilizer (9) (a 5 to 10% aqueous solution of chlorohexidine (trade name: "Hibiden"; manufactured by SUMITOMO PHARMACEUTICALS CO. LTD., Japan)) enclosed in said second bag-shaped member (8) was fallen down into the container. As a result, it is now possible that a disinfecting/sterilizing treatment of inner side of the container (1) including the medium is conducted without detaching the cover (2) from the container (1).

#### Example 8

(Use of Apparatus for Detecting Vibrio parahaemolyticus and Salmonella)

The same operation as in Example 7 was conducted except that the apparatuses for detecting Vibrio parahaemolyticus and for Salmonella prepared in Examples 2 and 3, respectively, were used to confirm the presence of food poisoning microorganism in cooking devices whereupon, in some cooking devices, presence of Vibrio parahaemolyticus or Salmonella was confirmed.

When those cooking devices were wiped with invert soap and washed with water by the same manner as in Example 7, the presence of Vibrio parahaemolyticus and Salmonella was not noted after said disinfecting treatment. Thus, it was now confirmed that Vibrio parahaemolyticus or Salmonella could be effectively removed by the above-mentioned

disinfecting treatment.

(Disinfecting/Sterilizing Treatment of Used Apparatus for Detecting Vibrio parahaemolyticus and Salmonella)

5 The second bag-shaped member (8) was broken by strongly compressing its cover (2) from outside using a compressing device (a "breaking device" in a broad clip-shaped one) (not shown) whereby a disinfectant/sterilizer (9) (a 5 to 10% aqueous solution of chlorohexidine (trade name: "Hibiden"; manufactured by SUMITOMO PHARMACEUTICALS CO. LTD., Japan)) enclosed in said second bag-shaped member (8) was fallen down into the container. As a result, it is now possible that a disinfecting/sterilizing treatment of inner side of the container (1) including the medium  
10 is conducted without detaching the cover (2) from the container (1).

#### Example 9

15 The same operation as in Example 7 was conducted for confirming the presence of food poisoning microorganism in cooking devices except that the detecting apparatuses having basic structures as shown in FIGS. 12 to 16 were used and that the screening media for Staphylococcus aureus, for Vibrio parahaemolyticus and for Salmonella mentioned in Examples 1, 2 and 3, respectively, and the antibiotic substances mentioned therein were jointly used whereupon, in some cooking devices, presence of Staphylococcus aureus, Vibrio parahaemolyticus or Salmonella was confirmed.

20 When those cooking devices were wiped with invert soap and washed with water by the same manner as in Example 7, the presence of Staphylococcus aureus, Vibrio parahaemolyticus and Salmonella was not noted after said disinfecting treatment. Thus, it was now confirmed that Staphylococcus aureus, Vibrio parahaemolyticus or Salmonella could be effectively removed by the above-mentioned disinfecting treatment.

(Disinfecting/Sterilizing Treatment of the Used Detecting Apparatus)

25 The second bag-shaped members (48, 64 (or 68), 88, 108) were broken by strongly compressing their covers (42, 62, 82, 102) from outside using a breaking function of the apparatus as shown in FIGS. 12 to 16 (such as 49, 461, 56, 53; 73, 74, 77; 93; 113) whereby a disinfectant/sterilizer (9) (a 5 to 10% aqueous solution of chlorohexidine (trade name: "Hibiden"; manufactured by SUMITOMO PHARMACEUTICALS CO. LTD., Japan)) enclosed in said second bag-shaped members (48, 64 (or 68), 88, 108) was fallen down into the container. As a result, it is now possible that a disinfecting/sterilizing treatment of inner side of the container (41, 61, 81, 101) including the medium is conducted without  
30 detaching the cover (42, 62, 82, 102) from the container (41, 61, 81, 101).

#### Example 10

35 By referring to FIGS. 17 to 33, the following materials were used to construct the microorganism-detecting apparatus having the following size:

Thus, the size from the bottom of the container (241) of the microorganism-detecting apparatus shown by FIGS. 17 and 18 to the top of a cap member (249) thereof (i.e. length of the microorganism-detecting apparatus) was about 201  
40 mm.

Container (241): a hollow cylinder having a bottom (inner diameter: about 9.0 mm; height: about 73 mm) made of polypropylene (thickness: about 1.0 mm) (At the outside near the opening, there were screw threads for connection.)

45 Rod-shaped part (member) (246): made of polypropylene; diameter: about 2.5 mm; length: about 77 mm

Microorganism-collecting end (246b): made of absorbent cotton; an end of "swab" having a maximum diameter of about 5 mm and a length of about 15 mm

Cover (243): The hollow cylindrical body (inner diameter: about 13.0 mm; length: about 96 mm; length of the engaging portion (259): about 10 mm) was made of polypropylene (thickness: about 1.0 mm). Inner side of the connecting part (259) had screw threads.  
50

Partition member (296): a disk with a diameter of about 13 mm made of polypropylene (thickness: about 1 mm). At its center a connecting means for a rod-shaped member of the microorganism-collecting part was placed. There were four perforated holes (through holes) in a shape of a fan. The partition member (296) was engaged with the connecting part (259) of the cover (243).

55 Cover (247): This (inner diameter: about 10 mm; length: about 57 mm; length of the head (245) (a connecting part with a cap (249)): about 13 mm) was made of polypropylene (thickness: about 1 mm). Inner side of the connecting part had screw threads. Ridge (or protruding portion) (297) placed on the inner side of the cover (243) (said protruding portion ranges to a protrusion (252)) and the groove (299) located at the outside of the cover (247) were



made in such a manner that they were engaged (or geared) each other whereby, when the cover (247) was inserted into the cover (243), they were tightly joined.

Partition member (235): This was made of polypropylene (thickness: about 1 mm) and its diameter was about 9 mm. There were four perforated holes (through holes) in a shape of a fan. The partition member (235) was engaged with an end of the side of the container (241) of the cover (247).

Cap material (249): made of polypropylene (thickness: about 1 mm). Diameter of the top side (561) which was opposite to the container (241) was about 15 mm. A cover (562) for a cap member was engaged therewith where diameter of the screw thread (257) was about 11 to 13 mm and diameter of the bottom (256) at the side of the container (241) was about 9 mm. Said screw threads (257) engaged with the grooves made inside of said connecting part (245). The screwed part formed at the inside of said connecting part (245) had a sufficient width (length of an axial direction of the cover (247)) so that the cap member (249) was able to be rotated and pushed thereinto.

First bag-shaped member (244): an ampule (inner diameter: about 9 mm; length: about 43 mm) made of glass (thickness: about 0.5 mm). It received, for example, the improved media as shown in Example 11 mentioned later and was made into an apparatus for detecting *Staphylococcus aureus*, *Vibrio parahaemolyticus*, *Salmonella* or *Escherichia coli* accordingly.

Second bag-shaped member (248): An ampule made of glass (thickness: about 0.5 mm) having inner diameter of about 9 mm and length of about 36 mm and receiving a disinfectant/bactericide as shown in Example 7 therein.

After manufacturing the microorganism-detecting apparatus having the above-mentioned constitution, it was sterilized by a sterilizer using ethylene oxide. The sterilization was conducted, for example, at the temperature of 30 to 50 °C in a gas consisting of about 20% of ethylene oxide and about 80% of carbon dioxide gas under the pressure of about 0.3 kg/m<sup>2</sup> for about five hours. The apparatus after the sterilization was placed in a bag made of polyethylene film (thickness: about 100 µm) evaporated with aluminum for shielding the light and stored until immediate before use. A medium of the following Example 11 was used by placing into the above-mentioned ampule (the first bag-shaped material (244)) followed by sealing.

Similarly were prepared the microorganism-detecting apparatuses by referring to FIGS. 34 to 51.

#### Example 11

##### (Preparation of a Microorganism-Detecting Apparatus Using Improved Medium)

(i) For a purpose of taking out from an ampule, a liquid medium is suitable and, from such a viewpoint, an improvement in media was conducted. Further, with an object of improving the specificity to the microorganism and of improving the operability of the microorganism-detecting apparatus, the composition for the medium was investigated whereby there will be no need of using a disk member (7) holding the antibiotics and an improvement was conducted for giving a selectivity to the medium. At the same time, investigation was made on pH indicators as well for such an improvement. As a result, the media having the following compositions were found to be appropriate:

##### (a) Improved Medium for Salmonella

##### (1) Composition

Base Medium:	
Tryptone (DIFCO)	5 g
Yeast extract (DIFCO)	3 g
L-(+)-Lysine monohydrochloride (WAKO; special grade)	10 g
Glucose (anhydrous) (WAKO; special grade)	1 g
Sodium chloride (WAKO; special grade)	8 g
Monopotassium dihydrogen phosphate (WAKO; sp gr)	1.6 g
Sodium thiosulfate pentahydrate (WAKO; special gr)	0.2 g
Ammonium iron(III) citrate (brown) (WAKO; extra pure gr)	0.3 g

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(continued)

Base Medium:	
Magnesium chloride hexahydrate (WAKO; special gr)	20.3 g
Bromocresol Purple (WAKO; special grade)	0.02 g
0.4% Malachite Green solution	30 ml
(Malachite Green oxalate (WAKO; special grade) was added to a small amount of pure alcohol, the mixture was well ground and pure water was added thereto to make the total volume 100 ml)	(0.4 g)

## (2) Method of Preparation

Each of the components in the above basic medium was weighed and 1000 ml of pure water was added thereto followed by well shaking and the resulting homogeneous floating liquid was mixed with 30 ml of a 0.4% Malachite Green solution followed by dissolving with heating (at 100 ° C for 20 minutes). The medium was adjusted to pH 5.3 to 5.7. After cooling, the medium was filtered through a filter of 0.2 µ.

## (b) Improved Medium for *Vibrio parahaemolyticus*

### (1) Composition

Base Medium:	
Salt-polymyxin broth (NISSUI SEIYAKU)	33 g
D-(-)-Mannitol (WAKO; special grade)	20 g
Sodium citrate dihydrate (WAKO; special grade)	8 g
Sodium thiosulfate pentahydrate (WAKO; special gr)	0.2 g
Bromocresol Purple (WAKO; special grade)	0.02 g

## (2) Method of Preparation

Each of the components for the above basic medium was weighed and 1000 ml of pure water was added thereto followed by well shaking to prepare a homogeneous floating liquid. The medium was adjusted to pH 7.0 to 7.4 and sterilized at 121 ° C for 15 minutes. Immediately after sterilization, it was cooled rapidly.

## (c) Improved Medium for *Escherichia coli* Group

### (1) Composition

Base Medium:	
Lauryl tryptose broth (DIFCO)	35.6 g
Lactose monohydrate (WAKO; special grade)	5 g
Bromthymol Blue (WAKO; special grade)	0.04 g

## (2) Method of Preparation

Each of the components for the above basic medium was weighed and 1000 ml of pure water was added thereto followed by well shaking to prepare a homogeneous floating liquid. The medium was adjusted to pH 6.75 to 7.25 and

sterilized at 121 ° C for 15 minutes. Immediately after sterilization, it was cooled rapidly.

(d) Improved Medium for Staphylococci

(1) Composition

Basic Medium:	
Tryptone (DIFCO)	10 g
Yeast extract (DIFCO)	5 g
D-(-)-Mannitol (WAKO; special grade)	10 g
Dipotassium monohydrogen phosphate (WAKO; sp gr)	5 g
Lithium chloride monohydrate (WAKO; special grade)	5.5 g
Glycine (WAKO; special grade)	16.5 g
Sodium pyruvate (WAKO; special grade)	12 g
Phenol Red (WAKO; special grade)	0.025 g
1% Aqueous solution of potassium tellurite	15 ml
Potassium tellurite (WAKO)	1 g
(Pure water (100 ml) was added to 1 g of potassium tellurite and the mixture was dissolved)	

(2) Method of Preparation

Each of the components in the above basic medium was weighed and 1000 ml of pure water was added thereto followed by well shaking to prepare a homogeneous floating liquid. The medium was adjusted to pH 7.25 to 7.75 and sterilized at 121 ° C for 15 minutes. When it was cooled down to 50 ° C or lower, 15 ml of 1% aqueous solution of potassium tellurite was added. After well mixing, the mixture was sterilized by filtering through a filter of 0.2 µ.

(ii) Measurement of Microorganism-Detecting Sensitivity and Specificity for Microorganism of Each Medium

Control strains, i.e. Salmonella (amount: 10<sup>9</sup> cells), Vibrio parahaemolyticus (amount: 10<sup>8</sup>), Escherichia coli group (amount: 10<sup>9</sup>) and Staphylococcus (amount: 10<sup>8</sup>), were dispersed in 1.5 ml of each medium to make the initial amount as shown in Tables 8 to 11 followed by incubating at 37 ° C for 24 hours in air.

Growth of each microorganism was judged by means of changes in color after the incubation. Incidentally, all of the above-mentioned control stains were the clinically separated strains and were identified by API 20 E (trade name; bioMerieux-Vitek Japan, Ltd., Japan) for Escherichia coli, Salmonella and Vibrio and by API Staph (trade name; bioMerieux-Vitek Japan, Ltd., Japan) for Staphylococcus.

In API 20 E (trade name; bioMerieux-Vitek Japan, Ltd., Japan), the following is exemplified in a positive rate table under the incubating conditions of 35 to 37 ° C for 24 or 48 hours:

They are Escherichia coli 1 and Escherichia coli 2 for Escherichia coli; Salmonella arizonae, Salmonella choleraesuis, Salmonella paratyphi A, Salmonella spp. and Salmonella typhi for Salmonella; and Vibrio alginolyticus, Vibrio cholerae, Vibrio hollisae, Vibrio metschnikovii, Vibrio mimicus, Vibrio parahaemolyticus and Vibrio vulnificus for Vibrio.

In API Staph (trade name; bioMerieux-Vitek Japan, Japan), the following is exemplified in a positive rate table under incubating condition of 35 to 37 ° C for 18 to 24 hours for Staphylococcus:

They are Staphylococcus aureus, Staphylococcus auricularis, Staphylococcus capitis, Staphylococcus caprae, Staphylococcus camosus, Staphylococcus chromogenes, Staphylococcus cohnii, Staphylococcus epidermidis, Staphylococcus haemolyticus, Staphylococcus hominis, Staphylococcus hyicus, Staphylococcus lentus, Staphylococcus lugdunensis, Staphylococcus saprophyticus, Staphylococcus schleiferi, Staphylococcus sciuri, Staphylococcus simulans, Staphylococcus warneri and Staphylococcus xylosus.

Table 7

(unit: CFU/ml)

Microorganism- Detecting Apparatus	Sensitivity	
	Before Improvement	After Improvement
Kit for <u>Staphylococcus</u>	$3.0 \times 10^3$	$1.9 \times 10^2$
Kit for <u>Salmonella</u>	$3.0 \times 10^4$	$1.4 \times 10^2$
Kit for <u>Vibrio</u> <u>parahaemolyticus</u>	$3.0 \times 10^5$	$3.1 \times 10^2$
Kit for <u>Escherichia</u> <u>coli group</u>	$3.0 \times 10^4$	$1.5 \times 10^2$

It is understood from Table 7 that, in the improved media, improvement in sensitivity was achieved in all microorganisms. A significant improvement was achieved especially in Vibrio parahaemolyticus. The following Tables 8 to 11 show the result of judgment of each of the media for the improved ones having the above-mentioned compositions as compared with the media before the improvement. In the tables, "Food Stamp" is a conventional name for a device for detecting microorganisms, for example, available from EIKEN KIZAI CO., LTD., Japan (trade name: "PETAN CHECK"), DENKA SEIKEN K.K., Japan (trade name: "DD CHECKER "SEIKEN") and NISSUI PHARMACEUTICAL CO., LTD (trade name: "SHOKUZAI CHECK "NISSUI"), and "San Coli" is a trade name for "Coli forms Detection Paper" available from SAN KAGAKU K.K., Japan.

TABLE 8

Kit for Staphylococcus							
Dilution	Rate: $10^X$	-3	-4	-5	-6	-7	-8
	Amount of Micro-organism	$10^5$	$10^4$	$10^3$	$10^2$	$10^1$	$10^0$
Media before Improvement	Change in Color	(+)	(+)	(+)	(±)	(-)	(-)
	Colony Numbers	Numerous	Numerous	Numerous	Negative	Negative	Negative
Media after Improvement	Change in Color	(+)	(+)	(+)	(+)	(-)	(-)
	Colony Numbers	Numerous	Numerous	Numerous	Numerous	Negative	Negative
Food Stamp		Numerous	Numerous	Numerous	Negative	Negative	Negative
"San-Coli"		(-)	(-)	(-)	(-)	(-)	(-)

TABLE 9

Kit for Salmonella							
Dilution	Rate: 10 <sup>X</sup>	-3	-4	-5	-6	-7	-8
	Amount of Micro-organism	10 <sup>6</sup>	10 <sup>5</sup>	10 <sup>4</sup>	10 <sup>3</sup>	10 <sup>2</sup>	10 <sup>1</sup>
Media before Improvement	Change in Color	(+)	(+)	(+)	(-)	(-)	(-)
	Colony Numbers	Numerous	Numerous	Numerous	Negative	Negative	Negative
Media after Improvement	Change in Color	(+)	(+)	(+)	(+)	(+)	(-)
	Colony Numbers	Numerous	Numerous	Numerous	Numerous	Numerous	Negative
Food Stamp		Numerous	Numerous	Numerous	Negative	Negative	Negative
"San-Coli"		(-)	(-)	(-)	(-)	(-)	(-)

TABLE 10

Kit for Vibrio parahaemolyticus							
Dilution	Rate: 10 <sup>X</sup>	-3	-4	-5	-6	-7	-8
	Amount of Micro-organism	10 <sup>6</sup>	10 <sup>5</sup>	10 <sup>4</sup>	10 <sup>3</sup>	10 <sup>2</sup>	10 <sup>1</sup>
Media before Improvement	Change in Color	(+)	(+)	(-)	(-)	(-)	(-)
	Colony Numbers	Numerous	Negative	Negative	Negative	Negative	Negative
Media after Improvement	Change in Color	(+)	(+)	(+)	(+)	(+)	(-)
	Colony Numbers	Numerous	Numerous	Numerous	Numerous	Numerous	Negative
Food Stamp		Numerous	Numerous	Numerous	Negative	Negative	Negative
"San-Coli"		(-)	(-)	(-)	(-)	(-)	(-)

TABLE 11

Kit for Escherichia coli group							
Dilution	Rate: 10 <sup>X</sup>	-3	-4	-5	-6	-7	-8
	Amount of Micro-organism	10 <sup>6</sup>	10 <sup>5</sup>	10 <sup>4</sup>	10 <sup>3</sup>	10 <sup>2</sup>	10 <sup>1</sup>
Media before Improvement	Change in Color	(+)	(+)	(+)	(-)	(-)	(-)
	Colony Numbers	Numerous	Numerous	Numerous	Negative	Negative	Negative
Media after Improvement	Change in Color	(+)	(+)	(+)	(+)	(+)	(-)
	Colony Numbers	Numerous	Numerous	Numerous	Numerous	Few	Negative
Food Stamp		Numerous	Numerous	Numerous	Negative	Negative	Negative
"San-Coli"		(-)	(-)	(-)	(-)	(-)	(-)

Staphylococcus aureus (MRSA), Staphylococcus aureus (MSSA), Staphylococcus epidermidis, Staphylococcus saprophyticus, Staphylococcus haemolyticus, Enterococcus sp., Bacillus sp., Salmonella typhimurium, Salmonella typhi, Proteus vulgaris, Proteus mirabilis, Citrobacter freundii, Citrobacter diversus, Klebsiella pneumoniae, Klebsiella oxytoca, Enterobacter aerogenes, Enterobacter cloacae, Serratia marcescens, Hafnia alvei, Morganella morganii, Vibrio parahaemolyticus, Vibrio fluvialis, Vibrio vulnificus, Aeromonas sp., Plesiomonas shigelloides, Escherichia coli (O-157 EHRC), Escherichia coli (O-55 EPEC), Escherichia coli (O-124 EIEC), Escherichia coli (O-25 ETRC), Pseudomonas sp., Acinetobacter sp., Flavobacterium sp., etc. were used and their detection was conducted whereupon good results were obtained. Relation between the microorganisms showing positive result and the changes in color of the medium is given in Table 12. It was noted that coloration was good and the resulting color had an excellent discriminating ability.

TABLE 12

Microorganism showing Positive Reaction and Color Change

Microorganism-Detecting Apparatus	Microorganisms showing Positive Reactions	Before Improvement		After Improvement	
		⊗Negative	Positive	⊗Negative	Positive
Kit for Detecting Staphylococcus	Staphylococcus aureus	pink	(weak) orange (strong) yellow	red	yellow
	Others	pink	yellow	red	
Kit for Detecting Salmonella	Salmonella	purple	dark purple	blue	purple
	genera Citrobacter and Enterobacter	purple	yellow	blue	purple
	Others	purple	yellow	blue	
Kit for Vibrio parahaemolyticus	genera Klebsiella, Proteus and E. coli group	purple	yellow	blue	
	Vibrio parahaemolyticus	bluish purple	yellowish green	purple	yellow
	Others	bluish purple	yellow	purple	yellow
Kit for Escherichia coli Group	genera Enterococcus and Serratia	bluish purple	yellow	purple	
	genera Klebsiella, Proteus and Citrobacter	bluish purple	yellow	purple	
	Escherichia coli group	purple	yellow	green	yellow
	Others	purple	yellow or white (transparent)	green	

Others: refer to the above-mentioned test microorganisms      ⊗Negative: the same color as before the test

An improved medium for *Escherichia coli* group mentioned in the above Example 11(i)(c) where Bromcresol Purple was substituted for Bromthymol Blue gave a good result in detecting the microorganisms in food and food materials. For



example, a good result was obtained in the detection of a microorganism in milk products by the use of a microorganism-detecting apparatus (containing said improved medium for Escherichia coli) constituted according to Example 10 (particularly by referring to FIGS. 34 to 51). The coloration was good and the resulting color had an excellent discriminating ability.

#### Example 12

By utilizing a microorganism-detecting apparatus as shown in FIG. 35 wherein a medium suitable for measuring numbers of viable cells is used, target microorganisms are cultured. After incubation for a determined period, the cultured medium is measured for turbidity. In the experimentation, it is checked whether or not the number of viable microorganism cells prior to the incubation can be estimated from the resultant turbidity data.

(1) To a medium for measuring the number of viable cells was added a solution of microorganisms with a predetermined concentration ( $10^8$  to  $10^1$  cells) and the medium was incubated at  $37^\circ\text{C}$  for a certain time (3, 5, and 6 hours, respectively).

#### Medium for measuring the number of viable cells

Composition per 1 liter of medium	
Meat extract	3.0 g
Casein peptone	15.0 g
Yeast extract	5.0 g
Glucose	1.0 g

(2) The cultured medium was diluted with a physiological saline solution to a 2-fold dilution. Then, said 2-fold diluted medium was applied to a turbidimeter (such as a device designed to measure the bacterial density in a liquid medium), McFarland turbidimeter (available from bioMerieux-Vitek Japan, Ltd., Japan; ATB1550) and measured for turbidity. The results are shown in FIGS. 71 and 72. FIG. 71 is the relation between the number of viable Staphylococcus aureus cells and the measured turbidity data. FIG. 72 is the relation between the number of viable Escherichia coli cells and the measured turbidity data.

In this experimentation, the number of the viable cells ranges from  $10^3$  to  $10^8$  cells after incubation for 5 to 6 hours and it is confirmed that there is a proportional relationship between the number of viable cells and turbidity data. Thus, it is understood that it is possible to estimate the number of viable microorganism cells prior to incubation by utilizing the microorganism-detecting apparatus according to the present invention.

Further, when a suitable incubation time and turbidity is selected, it would enable us to estimate the the number of viable microorganism cells prior to incubation. A concentration of medium for measuring the number of viable cells may be suitably selected in order to give a suitable turbidity value.

While the best mode for carrying out the invention has been described in detail, those familiar with the art to which this invention relates will recognize various alternative designs and embodiments for practicing the invention as defined by the following claims.

#### Claims

1. An apparatus for detecting microorganism, characterized in that, said apparatus comprises at least:

- (a) a container for holding a medium which is used for culturing the microorganism to be detected during the period of the incubation of said microorganism;
- (b) a microorganism-collecting part for taking microorganisms to be detected and transferring the collected microorganisms into the container;
- (c) a means for allowing us to holding the medium for culturing the microorganism to be detected, in a noncontact manner with the microorganism-collecting part (b), until the incubating stage of the microorganism; and

(d) a means for disinfecting the medium after incubation.

2. The apparatus for detecting microorganism according to claim 1 wherein the means (d) (means for disinfecting the medium after incubation) includes means for allowing a disinfectant to be held, without contact with the microorganism, until the completion of the incubation for the microorganism to be detected.

3. The apparatus for detecting microorganism according to claim 1 or 2 wherein the medium and the disinfectant are made in such a manner that they can unitedly contact each other, by the action of a force applied from outside, after incubation of the microorganism.

4. The apparatus for detecting microorganism according to any of claims 1 to 3 wherein the microorganism-collecting part (b) and the medium used for culturing the microorganism to be detected are made in such a manner that they can unitedly contact each other, by the action of a force applied from outside, during the incubation of the microorganism.

5. The apparatus for detecting microorganism according to any of claims 1 to 4 wherein said apparatus contains:

(a) a hollow container having an opening at one of the ends;

(b) a microorganism-collecting part located in said hollow container;

(c) a medium for culturing the microorganism, said medium being located so as to contact with said microorganism-collecting part during the incubation of the microorganism; and

(d) a disinfectant being located so as to contact with the medium for culturing the microorganism after the incubation of the microorganism.

6. The apparatus for detecting microorganism according to any of claims 1 to 5 wherein an antibiotic substance is added to said medium.

7. The apparatus for detecting microorganism according to any of claims 1 to 6 wherein an antibiotic substance is contained, at least a part of said antibiotic substance coming unitedly into contact with the medium, by the action of an external force, during the incubation of the microorganism, and at least said antibiotic substance and the medium being located in the storage container in a noncontact manner.

8. The apparatus for detecting microorganism according to any of claims 1 to 7 wherein microorganisms are detected through incubating the microorganism in a medium and observing the changes in the medium, characterized in that said apparatus comprises

(i) a microorganism-collecting part,

(ii) an antibiotic substance,

(iii) a medium and

(iv) a disinfectant,

wherein at least a part of (i) the microorganism-collecting part, (ii) the antibiotic substance and (iii) the medium can unitedly contact each other, by the action of the force from outside, during the incubation of the microorganism, at least the above-mentioned antibiotic substance (ii) and the above-mentioned medium (iii) being located in a storage container in a noncontact manner, and

wherein, after incubation of the microorganism, at least the incubated microorganism and the disinfectant (iv) can unitedly contact each other by the action of the force from outside, at least the above-mentioned disinfectant (iv) and the above-mentioned medium (iii) being located in the storage container in a noncontact manner.

9. The apparatus for detecting microorganism according to any of claims 1 to 5 and 7 to 8 wherein said antibiotic substance is

(1) located in the above-mentioned container,

(2) given to the above mentioned microorganism-collecting part or

(3) coated on the inner wall of the above-mentioned container.

10. The apparatus for detecting microorganism according to any of claims 1 to 5 and 7 to 9 wherein said antibiotic substance is given to other materials located in said hollow container.

11. The apparatus for detecting microorganism according to any of claims 1 to 5 and 7 to 9 wherein said microorganism-collecting part has a multiple structure containing at least an inside material and an outside material and said antibiotic substance is located in said inside material.
- 5 12. The apparatus for detecting microorganism according to any of claims 1 to 11 wherein said medium contains a substance capable of changing its color according to the change caused by growth of the microorganism.
13. The apparatus for detecting microorganism according to any of claims 1 to 12 wherein said container has a cover for tight closing and said medium is located in said cover in such a manner that it is enclosed in a bag-shaped member.  
10
14. The apparatus for detecting microorganism according to claim 13 wherein a perforated material is located between said bag-shaped member and said hollow container.
- 15 15. The apparatus for detecting microorganism according to claim 14 wherein a guide member which can promote a falling-down of the medium along said microorganism-collecting part is located under said perforated material.
16. The apparatus for detecting microorganism according to any of claims 1 to 15 wherein said apparatus has an incubation selectivity to at least one microorganism selected from the group consisting of enteropathogenic Escherichia coli, Staphylococcus aureus, Vibrio parahaemolyticus and Salmonella.  
20
17. The apparatus for detecting microorganism according to any of claims 1 to 16 wherein said apparatus has a structure for allowing us to disinfect the medium used for the incubation under a tightly closed state.
- 25 18. The apparatus for detecting microorganism according to any of claims 1 to 17 wherein said apparatus has a structure for allowing us to conduct at least
  - (i) addition of a medium into a container,
  - (ii) incubation,
  - 30 (iii) visual detection of the microorganism cells, and
  - (iv) disinfecting treatment of the incubated medium under a tightly closed state after collecting treatment of the microorganism by the microorganism-collecting part.
- 35 19. An apparatus for detecting microorganism, characterized in that a cover is composed of:
  - (a) a first cover-constituting member which constitutes a first section for receiving a first bag-shaped member,
  - (b) a second cover-constituting member which constitutes a second section for receiving a second bag-shaped member, and
  - 40 (c) a cap member being located at one end of said second cover-constituting member and at the opposite side of the fitting side (of said second cover-constituting member) for said first cover-constituting member and rotatably screwed into said second cover-constituting member;  
wherein, at or near each end of the first and second cover-constituting members, each end being located at the side of a container having a space for culturing the microorganism by receiving a medium, either a partition is formed or a member for the partition is disposed for keeping each of said bag-shaped members;
  - 45 said first cover-constituting member being slidably engaged with one end of said second cover-constituting member wherein said first bag-shaped member can be broken to release the medium contained therein by sliding said second cover-constituting member to the side of said first cover-constituting member by a force from outside (external force);  
wherein said second bag-shaped member can be broken to release a disinfectant contained therein by  
50 rotating said cap member by a force from outside (external force), and  
wherein one or more through holes are formed in said partition or said partition member in a manner that the medium and the disinfectant can be communicated with said container.
- 55 20. The apparatus for detecting microorganism according to any of claims 1 to 19, comprising a medium selected from the group consisting of:
  - (a) a medium for Salmonella having a composition substantially containing an appropriate amount, such as 3 to 7 g. of tryptone, an appropriate amount, such as 1 to 6 g. of yeast extract, an appropriate amount, such as

5 to 15 g, of lysine, an appropriate amount, such as 0.5 to 2 g, of glucose, an appropriate amount, such as 7 to 9 g, of sodium chloride, an appropriate amount, such as 1.0 to 2.0 g, of monopotassium dihydrogen phosphate, an appropriate amount, such as 0.1 to 0.3 g, of sodium thiosulfate, an appropriate amount, such as 0.2 to 0.4 g, of ammonium iron citrate, an appropriate amount, such as 15 to 25 g, of magnesium chloride, an appropriate amount, such as 27 to 33 ml, of 0.4% Malachite Green solution and an appropriate amount, such as 0.01 to 0.03 g, of Bromocresol Purple per 1,000 ml of the medium (pH of the medium being about 5.3 to 5.7);

(b) a medium for *Vibrio parahaemolyticus* having a composition substantially containing an appropriate amount, such as 25 to 40 g, of a salt polymyxin broth (where the salt polymyxin broth contains an appropriate amount of yeast extract, an appropriate amount of peptone, an appropriate amount of sodium chloride and an appropriate amount polymyxin B), an appropriate amount, such as 15 to 25 g, of mannitol, an appropriate amount, such as 5 to 10 g, of sodium citrate, an appropriate amount, such as 0.1 to 0.3 g, of sodium thiosulfate and an appropriate amount, such as 0.01 to 0.03 g, of Bromocresol Purple per 1,000 ml of the medium (pH of the medium being about 7.0 to 7.4);

(c) a medium for *Escherichia coli* group having a composition substantially containing an appropriate amount, such as 26 to 43 g, of lauryl sulfate broth (the lauryl sulfate broth contains an appropriate amount of tryptose, an appropriate amount of lactose, an appropriate amount of monopotassium dihydrogen phosphate, an appropriate amount of dipotassium monohydrogen phosphate, an appropriate amount of sodium chloride and an appropriate amount of sodium lauryl sulfate), an appropriate amount, such as 3 to 8 g, of lactose and an appropriate amount, such as 0.03 to 0.05 g, of Bromthymol Blue or Bromocresol Purple per 1,000 ml of the medium (pH of the medium being about 6.75 to 7.25); and

(d) a medium for *Staphylococcus* having a composition substantially containing an appropriate amount, such as 5 to 15 g, of tryptone, an appropriate amount, such as 2 to 8 g, of yeast extract, an appropriate amount, such as 5 to 15 g, of mannitol, an appropriate amount, such as 2 to 8 g, of dipotassium monohydrogen phosphate, an appropriate amount, such as 5 to 6 g, of lithium chloride, an appropriate amount, such as 12 to 20 g, of glycine, an appropriate amount, such as 10 to 14 g, of sodium pyruvate, an appropriate amount, such as 12 to 18 ml, of 1% aqueous solution of potassium tellurite and an appropriate amount, such as 0.02 to 0.03 g, of Phenol Red per 1,000 ml of the medium (pH of the medium being about 7.25 to 7.75).

21. The apparatus for detecting microorganism according to any of claims 1 to 5 wherein microorganisms are detected through incubating the microorganism in a medium and observing the changes in the medium, characterized in that said apparatus comprises

- (i) a microorganism-collecting part,
- (ii) a medium and
- (iii) a disinfectant,

wherein at least a part of (i) the microorganism-collecting part and (ii) the medium can unitedly contact each other, by the action of the force from outside, during the incubation of the microorganism, the above-mentioned microorganism-collecting part (i) and the above-mentioned medium (ii) being at least located in a storage container in a noncontact manner, and

wherein, after incubation of the microorganism, at least the incubated microorganism and the disinfectant (iii) can unitedly contact each other by the action of the force from outside, at least the above-mentioned disinfectant (iii) and the above-mentioned medium (ii) being located in the storage container in a noncontact manner.

22. The apparatus for detecting microorganism according to any of claims 1 to 21 wherein said container comprises a cover for closing an opening, said cover accommodating a bag-shaped member which encloses said medium.
23. The apparatus for detecting microorganism according to any of claims 1 to 22 wherein said container comprises a cover member for closing an opening, said cover accommodating a bag-shaped member which encloses said disinfectant.
24. The apparatus for detecting microorganism according to any of claims 1 to 23 wherein said cover has a structure for allowing it to hold and carry at least
- (i) a first bag-shaped member containing a medium, and
  - (ii) a second bag-shaped member containing a disinfectant independently each other.

25. The apparatus for detecting microorganism according to any of claims 1 to 24 wherein said cover comprises at

least

- (i) a first cover-constituting member accommodating the first bag-shaped member, and
- (ii) a second cover-constituting member accommodating the second bag-shaped member.

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26. The apparatus for detecting microorganism according to any of claims 1 to 25, comprising

a means for protection and engagement, being disposed in said first and second cover-constituting members so as not break the first bag-shaped member by moving the second cover-constituting member prior to use.

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27. The apparatus for detecting microorganism according to any of claims 1 to 26, wherein said second cover-constituting member is equipped with a cap member and a means for protection and engagement is disposed in said second cover-constituting member and said cap member so as not to break the second bag-shaped member by moving the cap member prior to use.

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28. The apparatus for detecting microorganism according to claim 26 or 27, wherein said means for protection and engagement is a combination of a stopper and a guide.

29. A method for quantitatively measuring the number of viable microorganism cells in a sample or specimen through using the apparatus for detecting microorganism according to any of claims 1 to 19 and 21 to 28.

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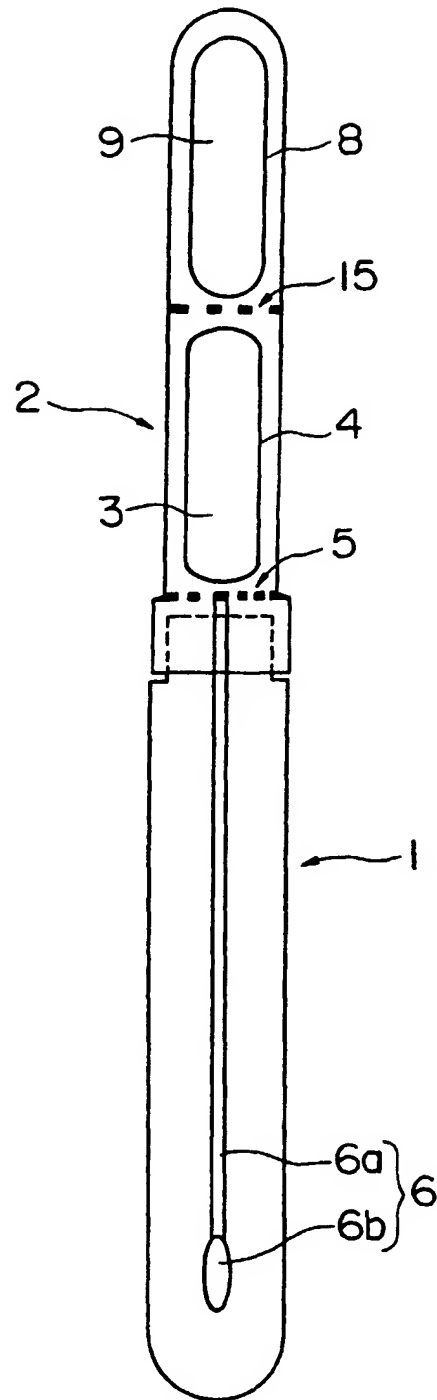


FIG. 1

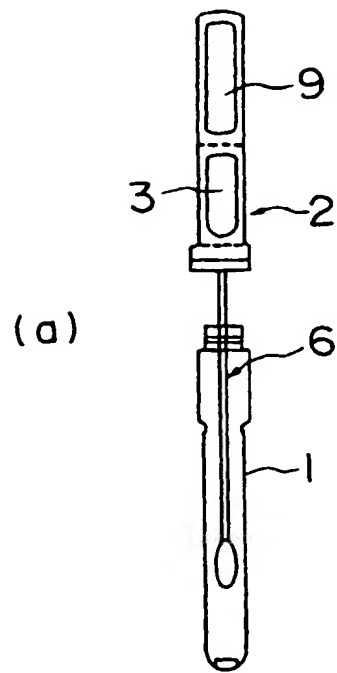


FIG. 2

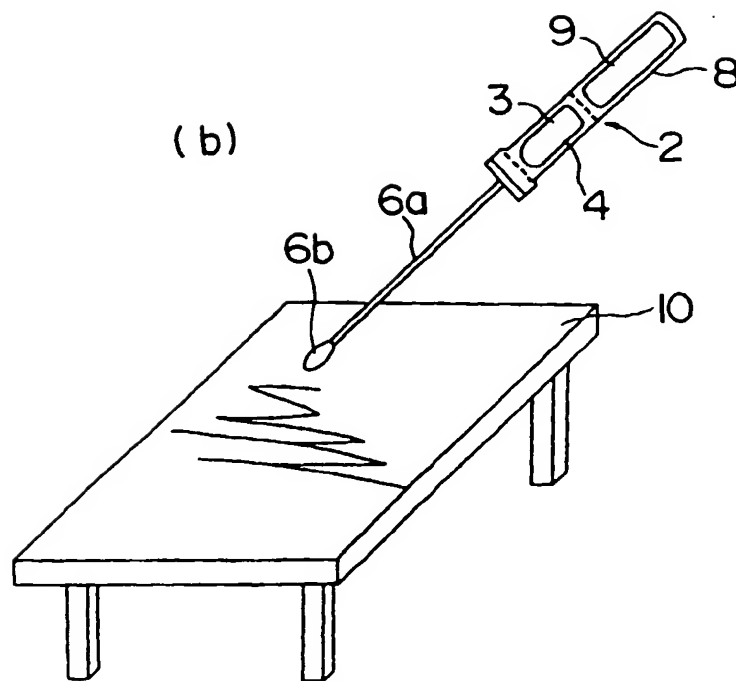
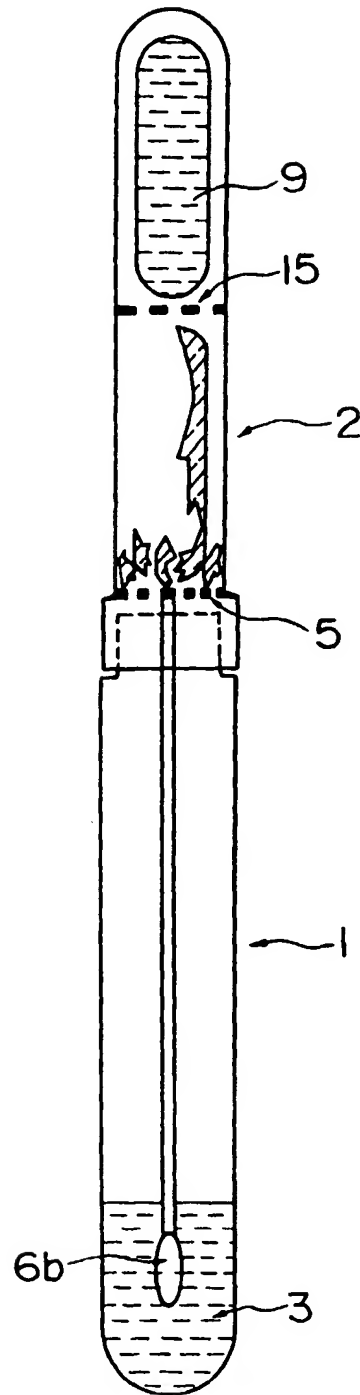




FIG. 3



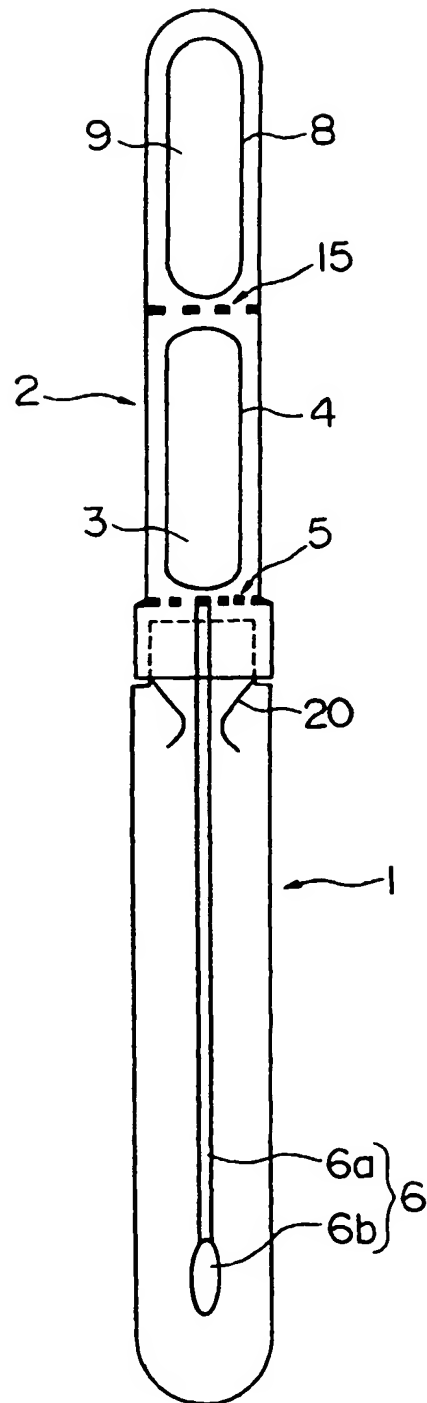
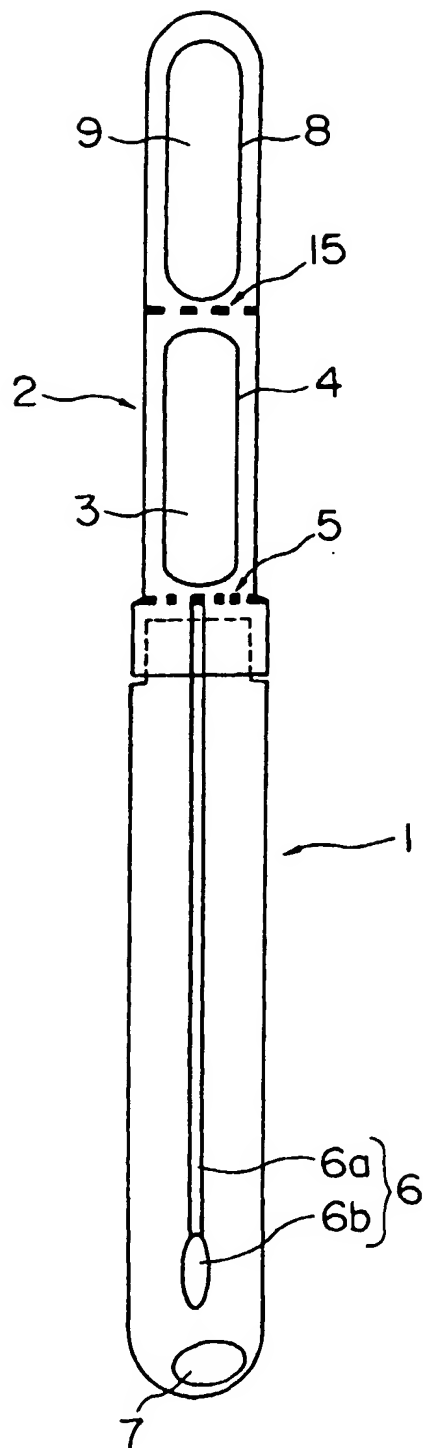
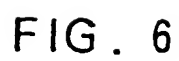


FIG. 4

FIG. 5





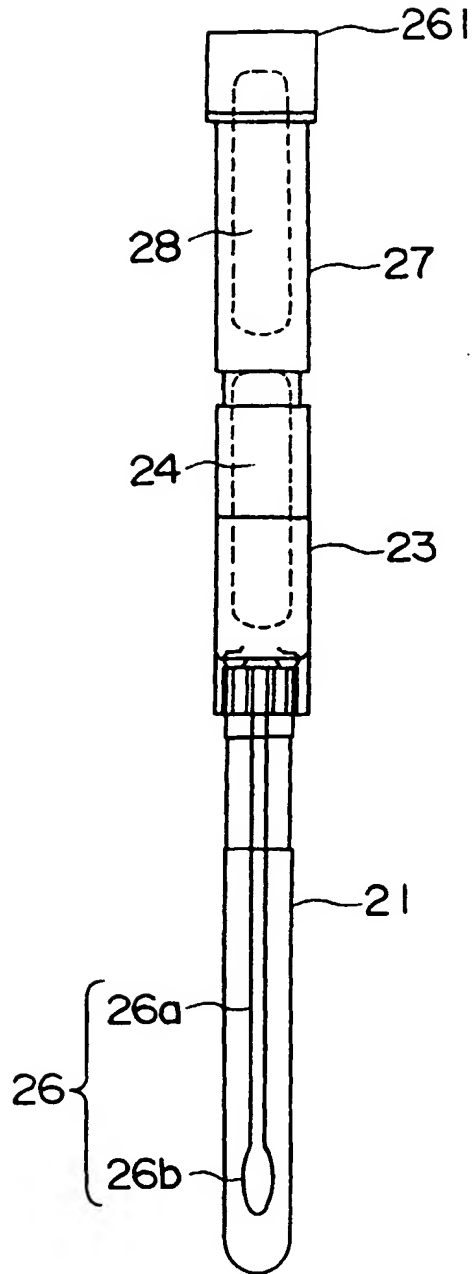


FIG. 7

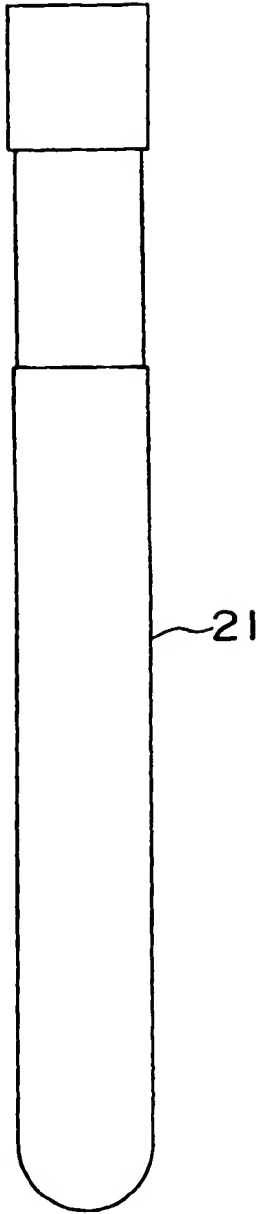


FIG. 8

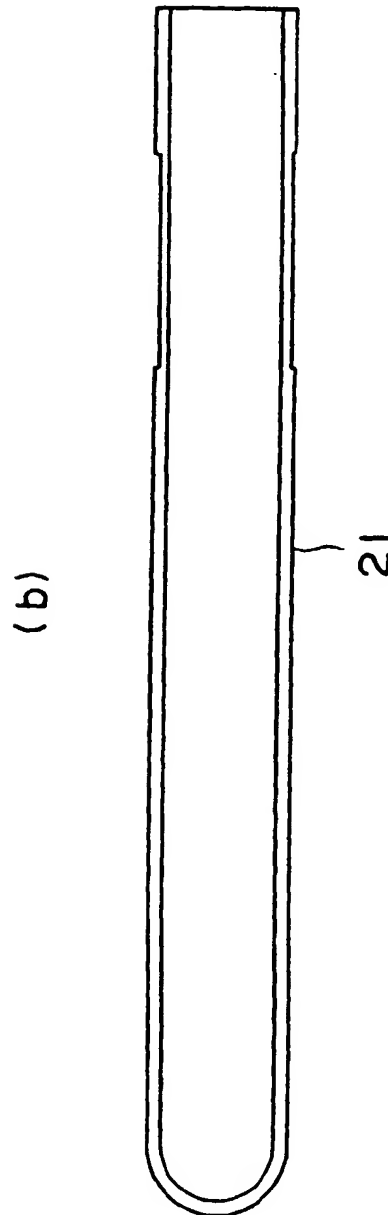
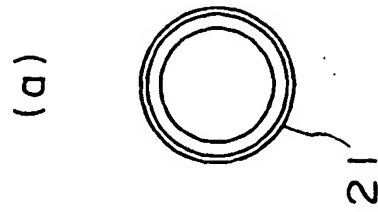


FIG. 9

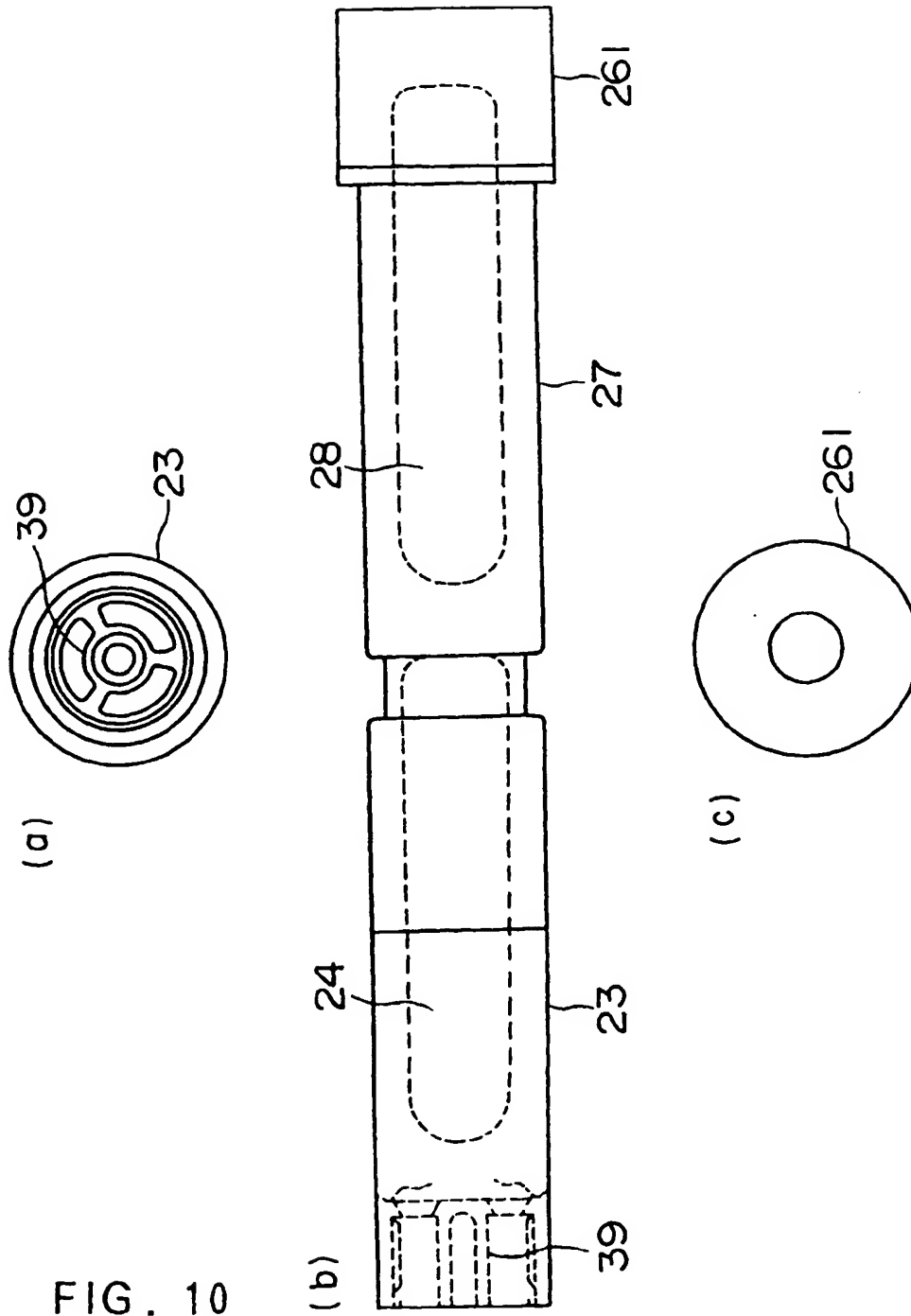


FIG. 10



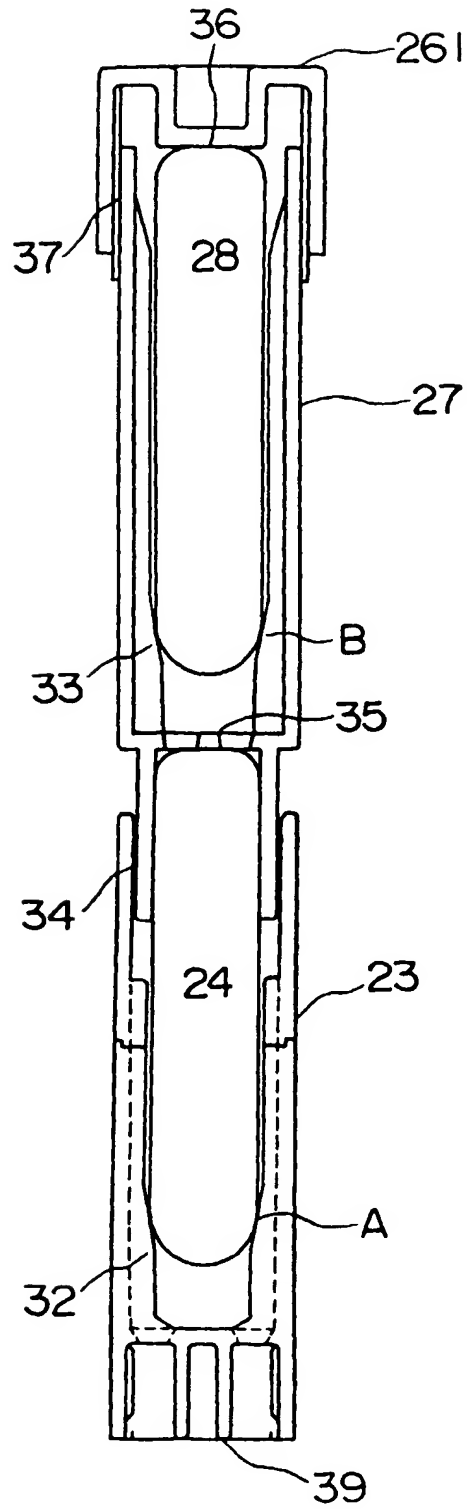


FIG. 11

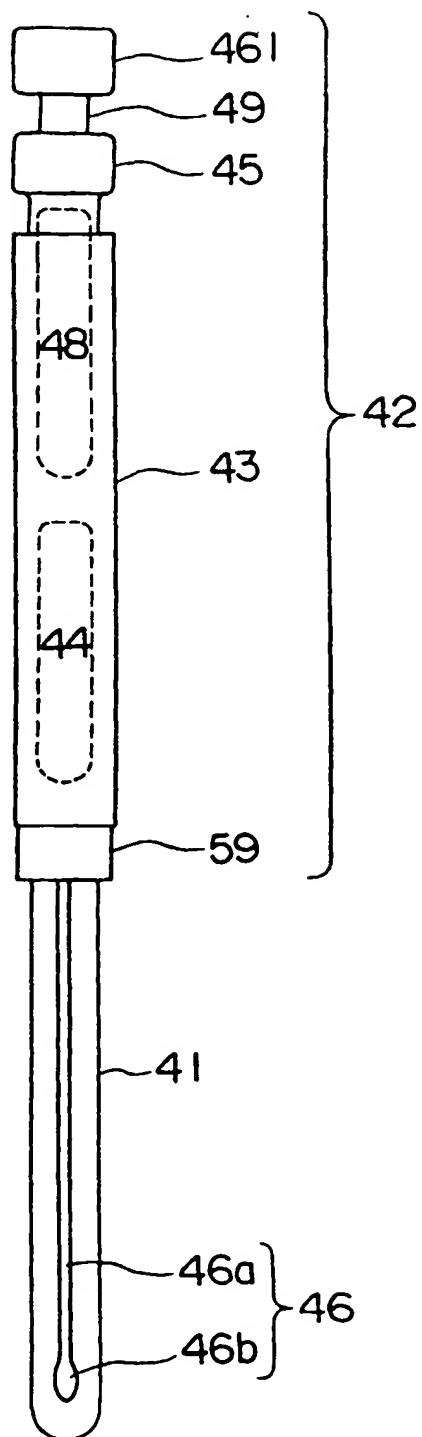


FIG. 12

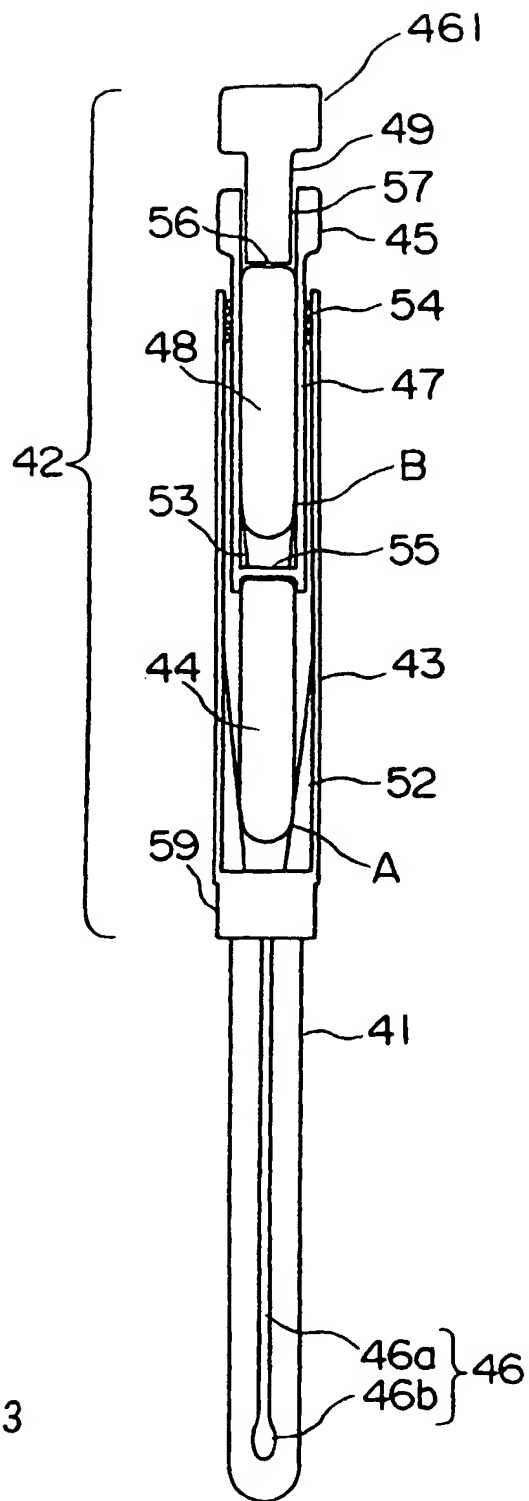


FIG. 13

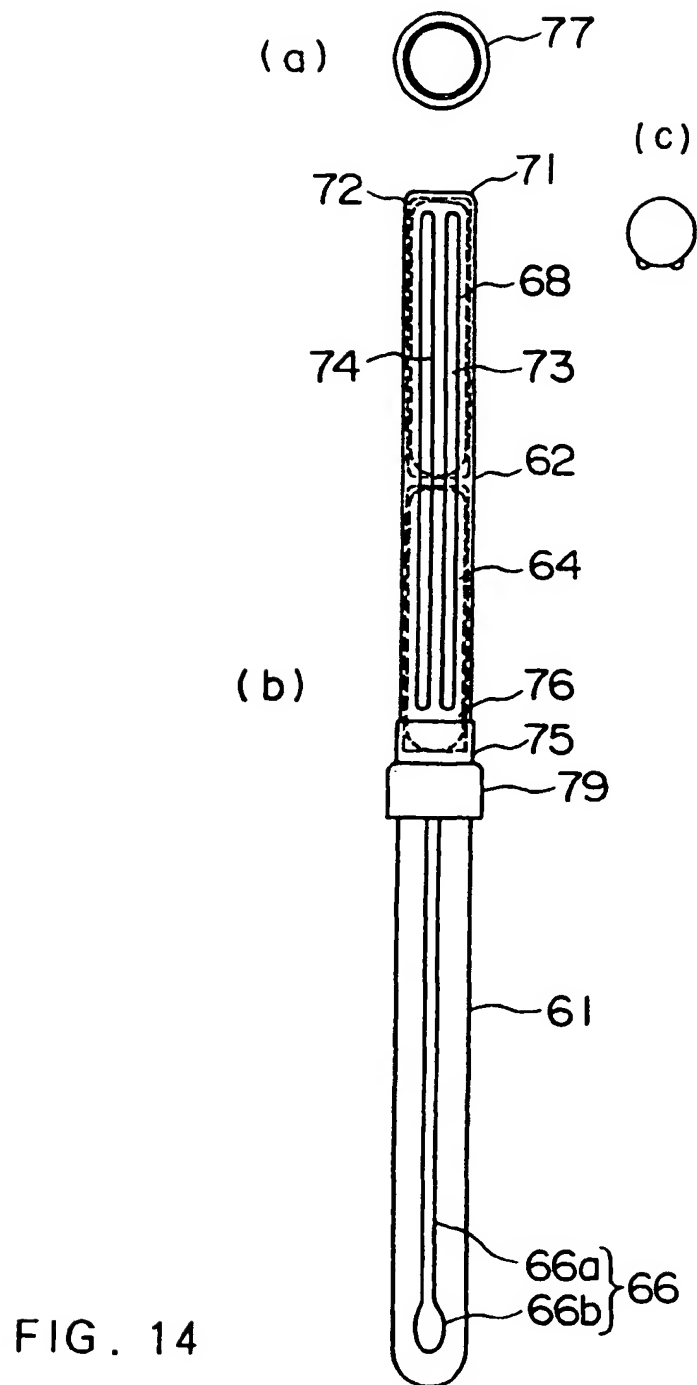


FIG. 14

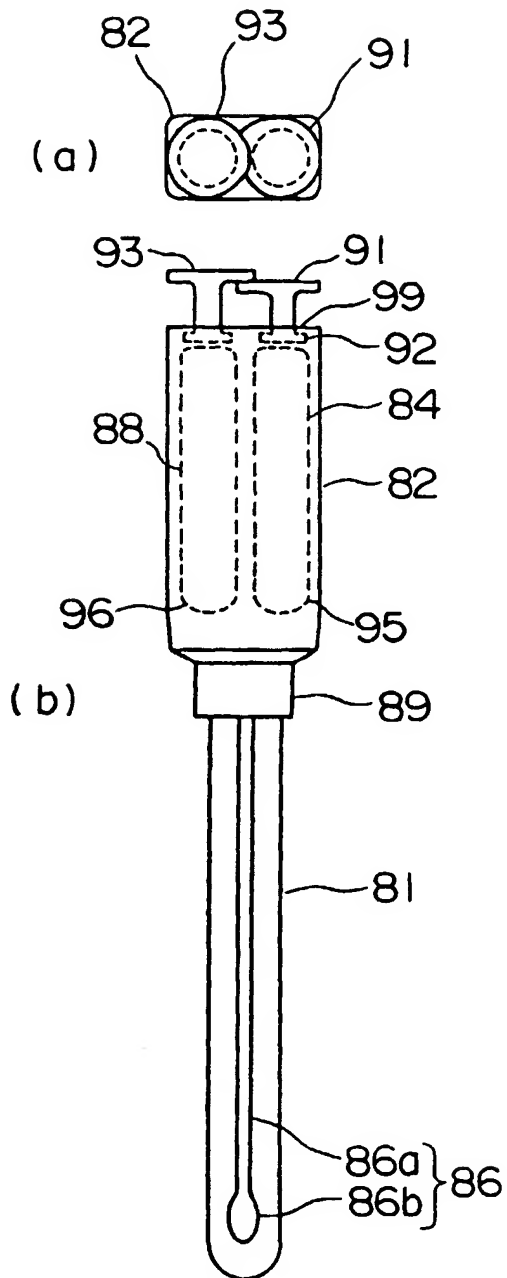
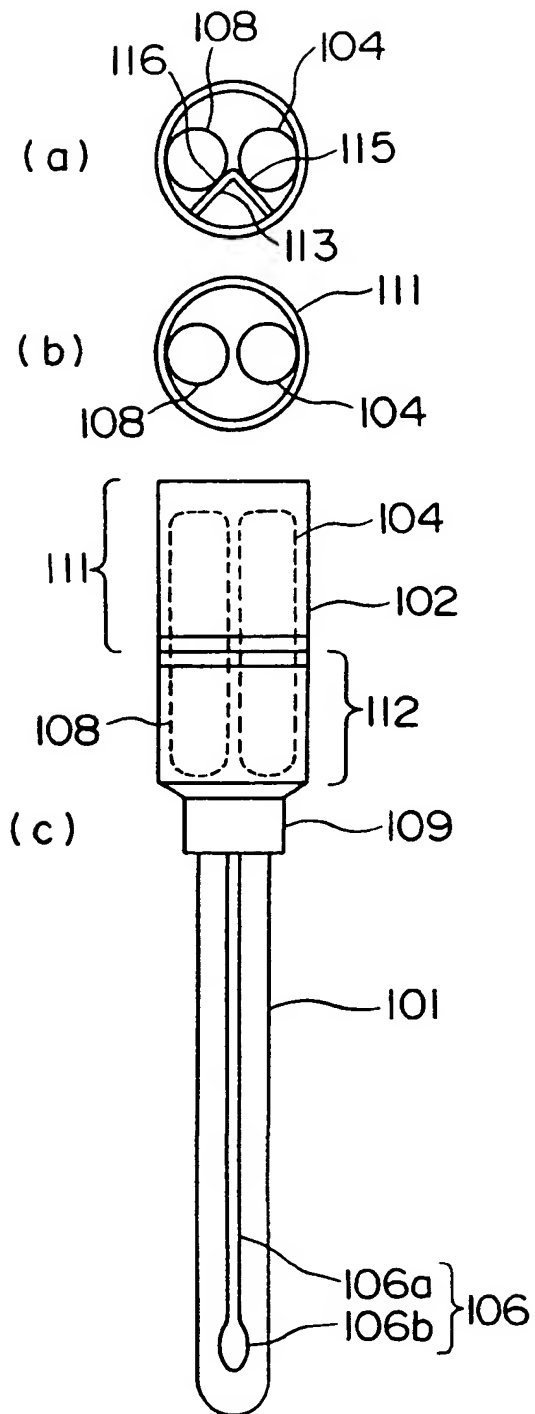


FIG. 15



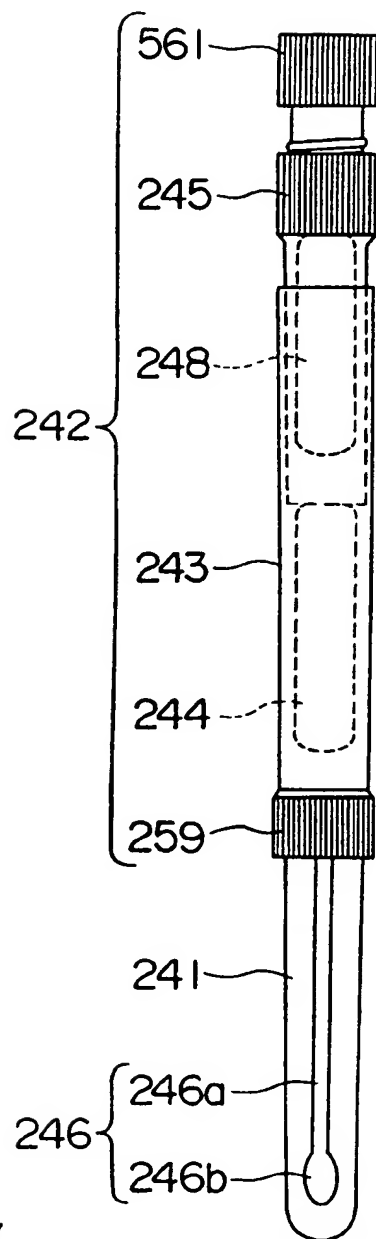


FIG. 17

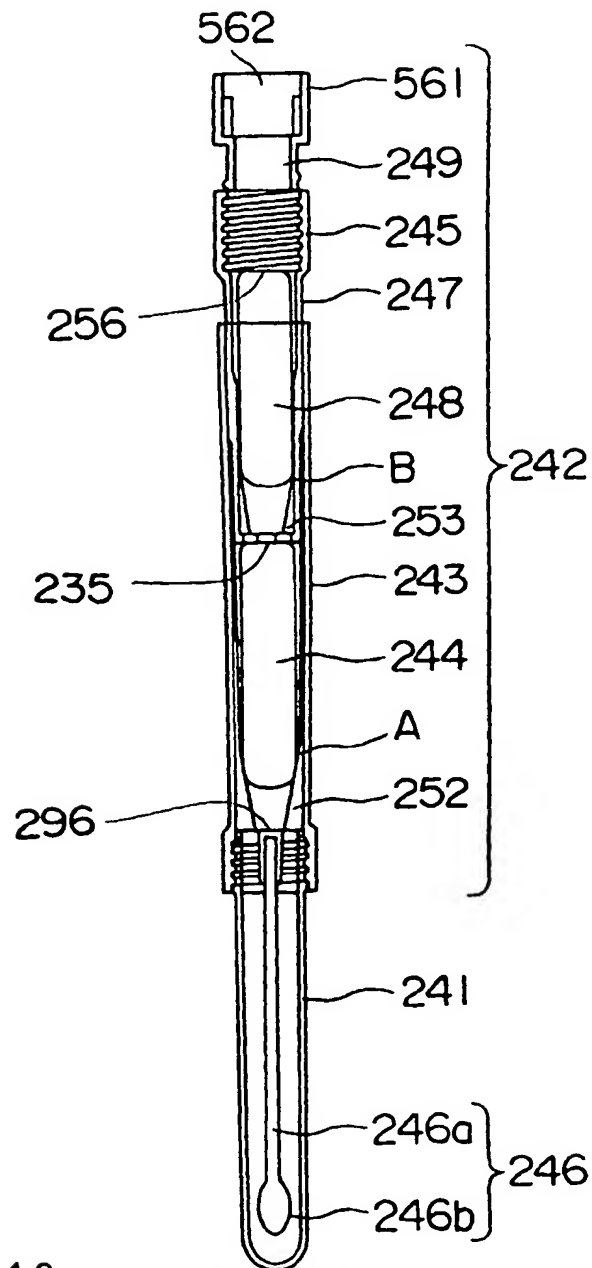
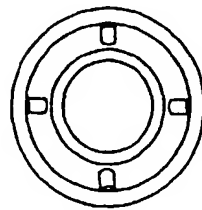
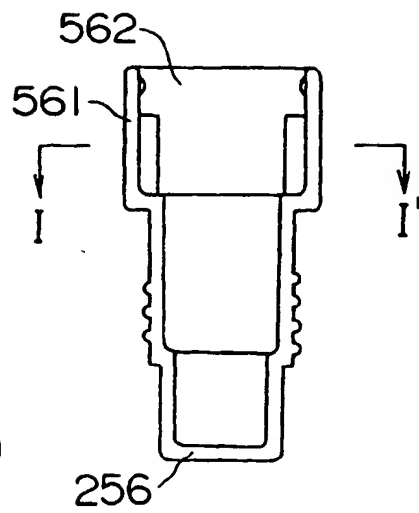
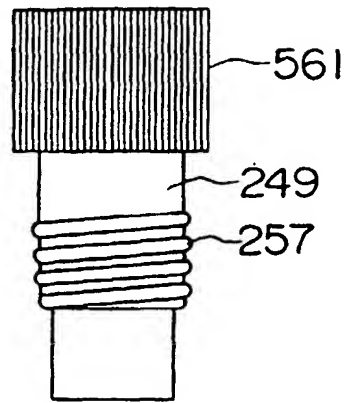


FIG. 18





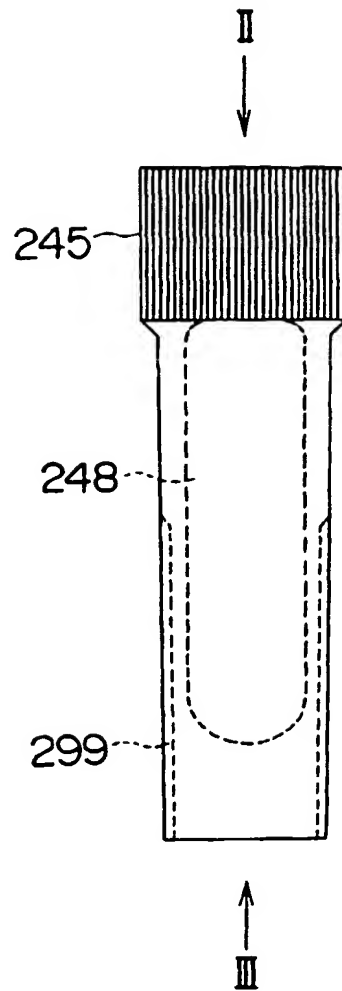


FIG. 22

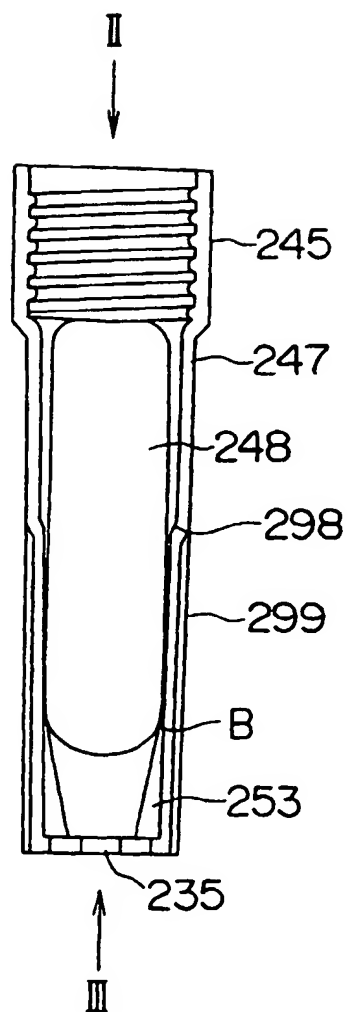


FIG. 24

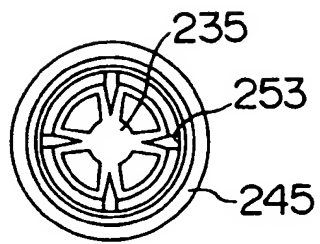
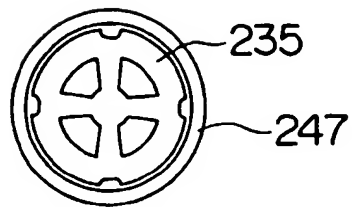


FIG. 25



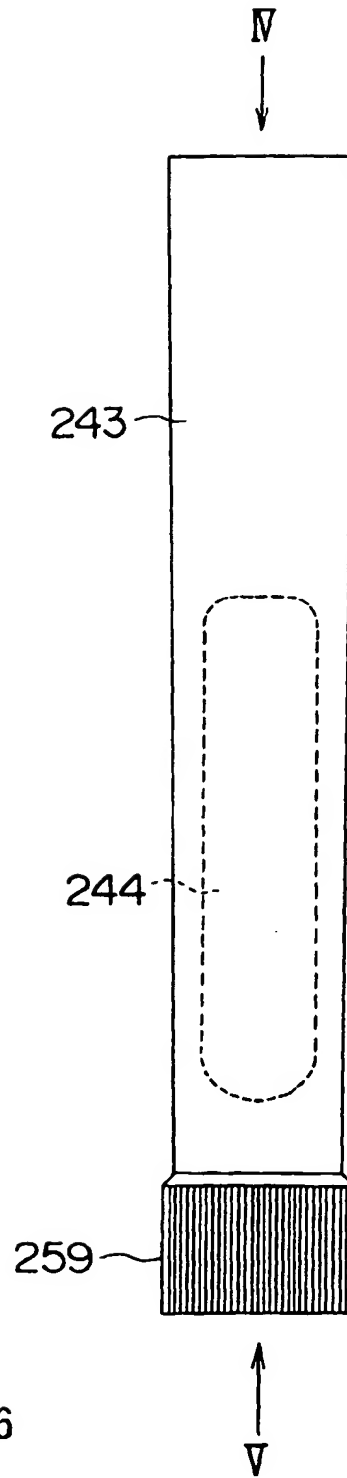


FIG. 26

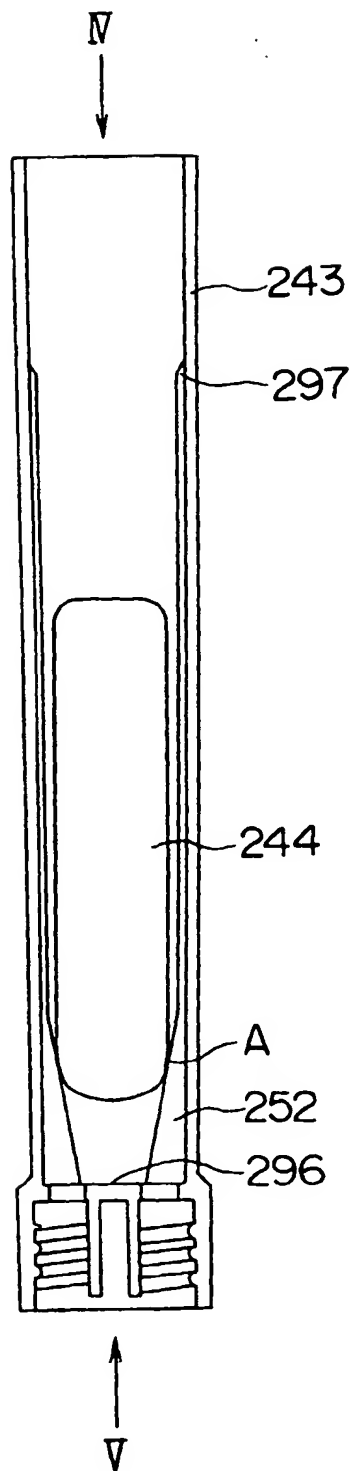


FIG. 27

FIG. 28

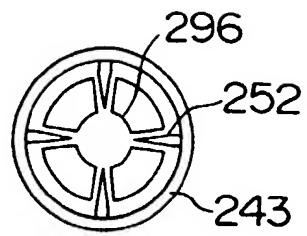
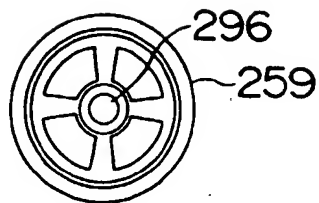


FIG. 29



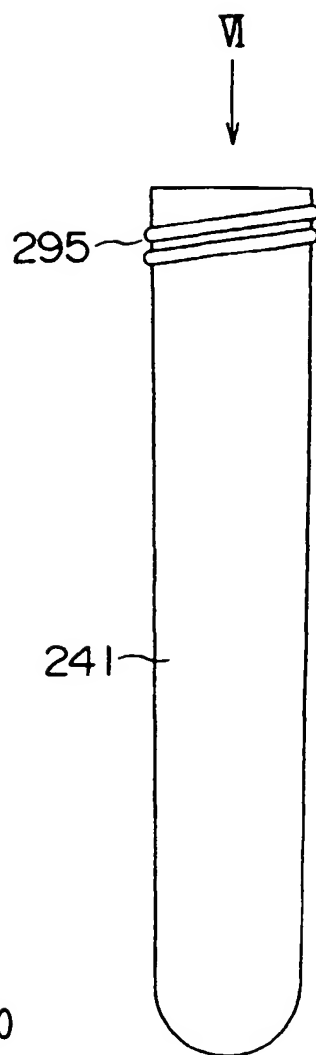


FIG. 30



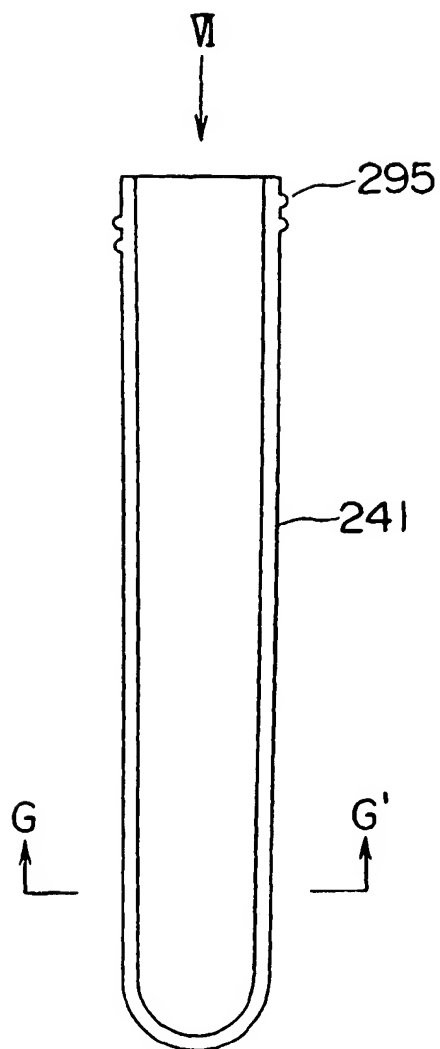


FIG. 31

FIG. 32

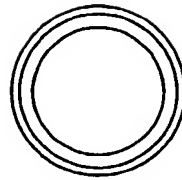
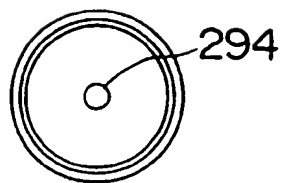


FIG. 33



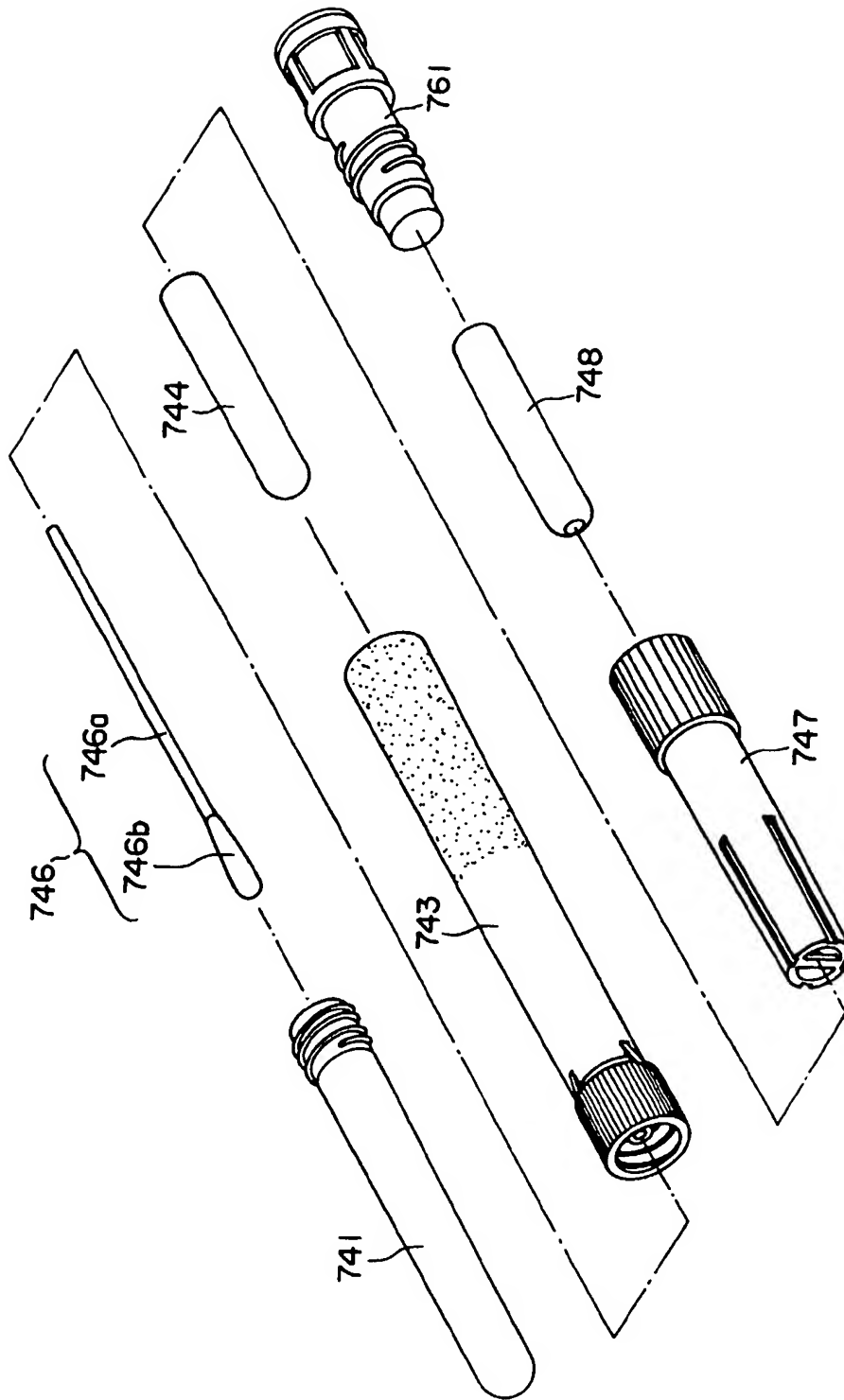


FIG. 34

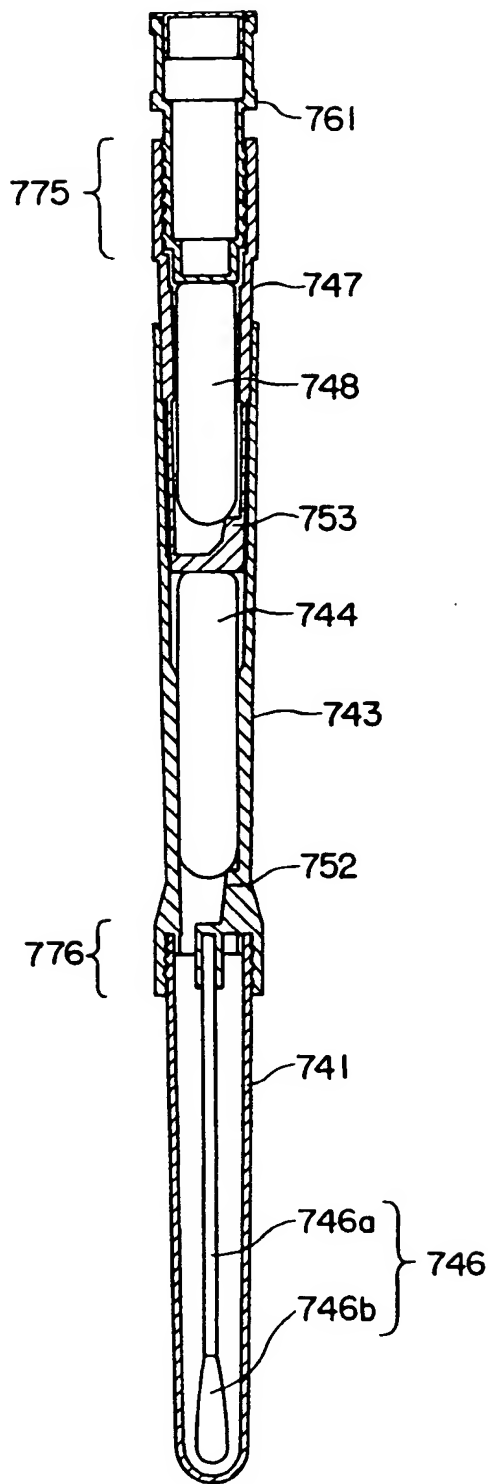


FIG. 35

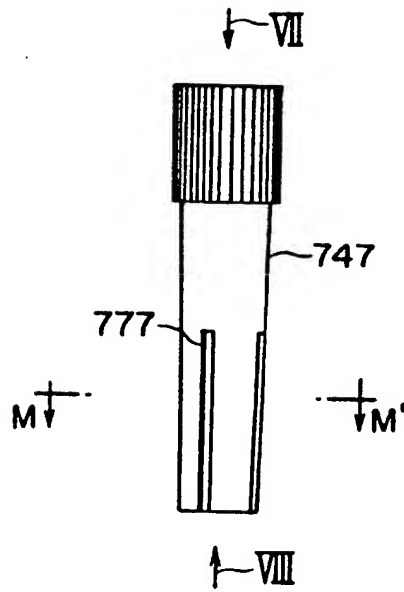


FIG. 36

FIG. 37

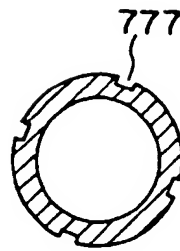


FIG. 38

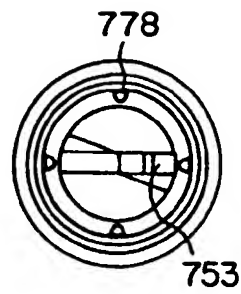
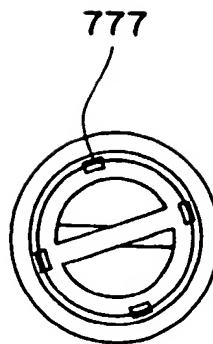


FIG. 39



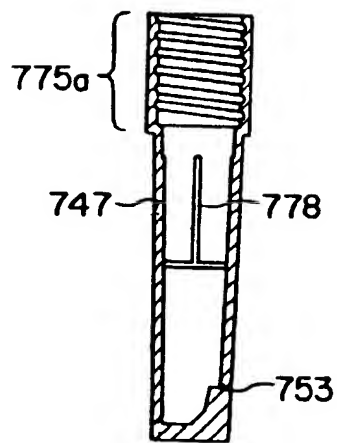


FIG. 40

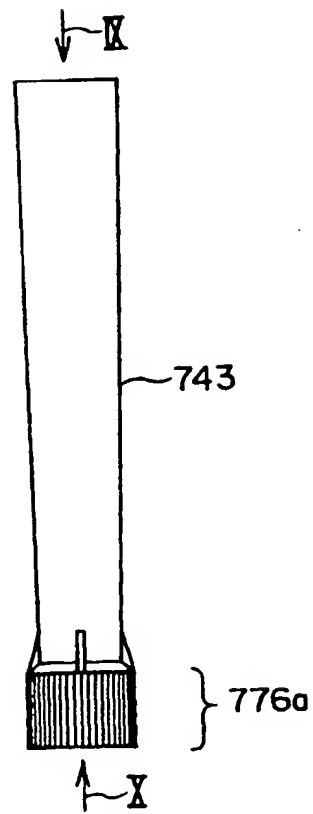


FIG. 41



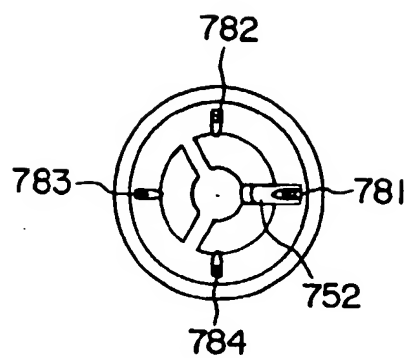


FIG. 42

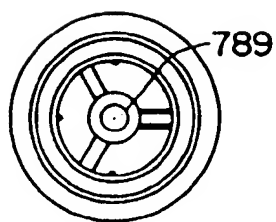


FIG. 43

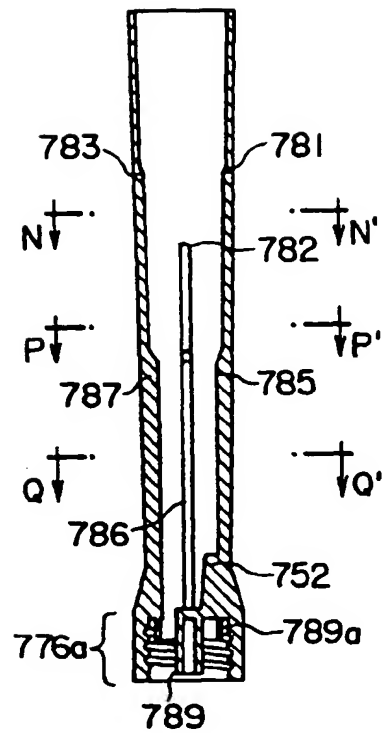


FIG. 44

FIG. 45

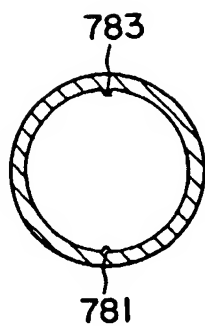


FIG. 46

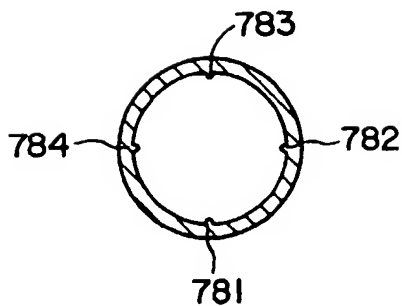
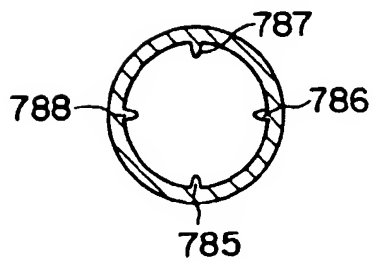


FIG. 47



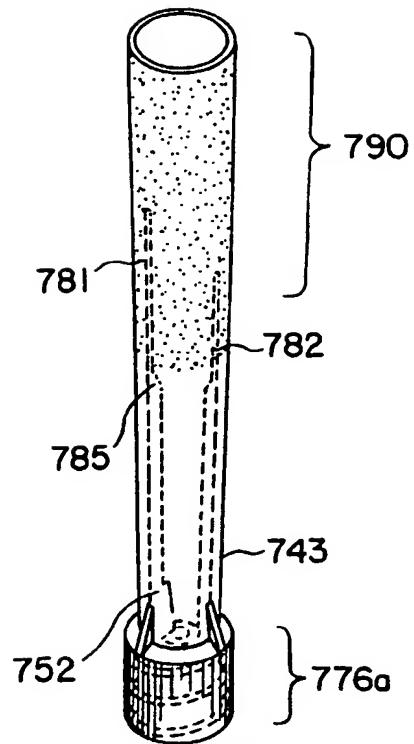


FIG. 48

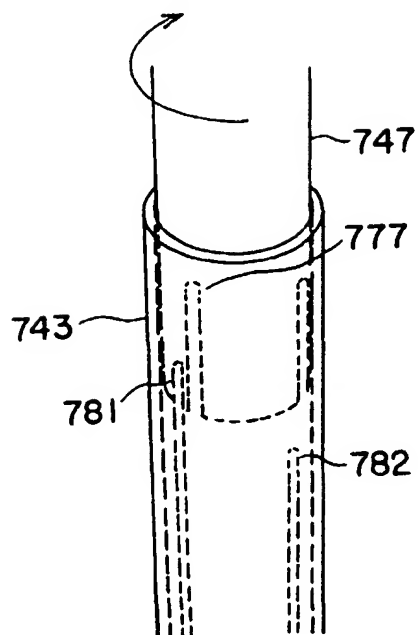


FIG. 49

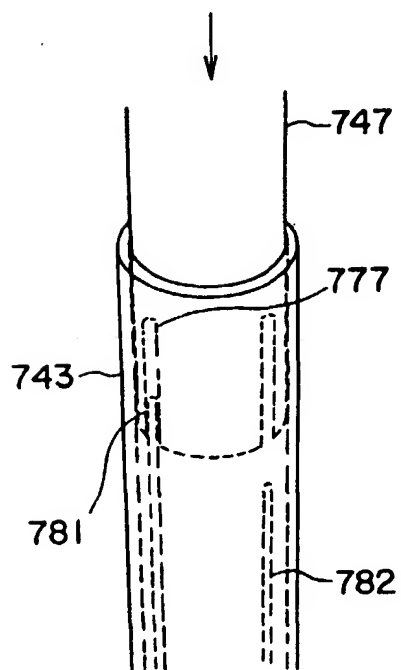


FIG. 50

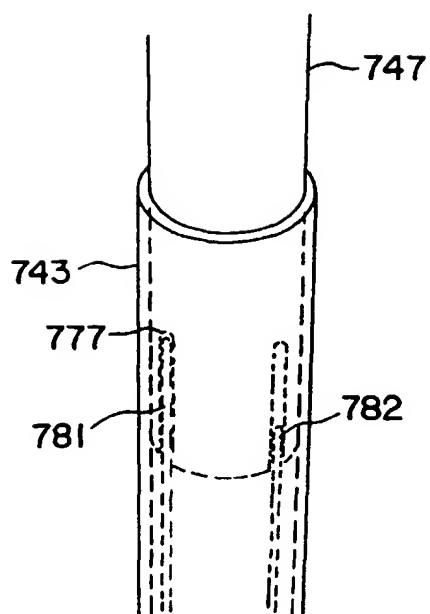


FIG. 51

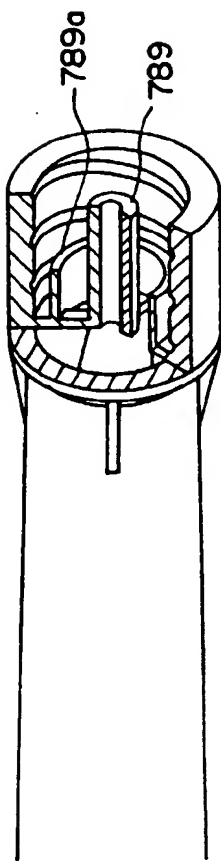


FIG. 52

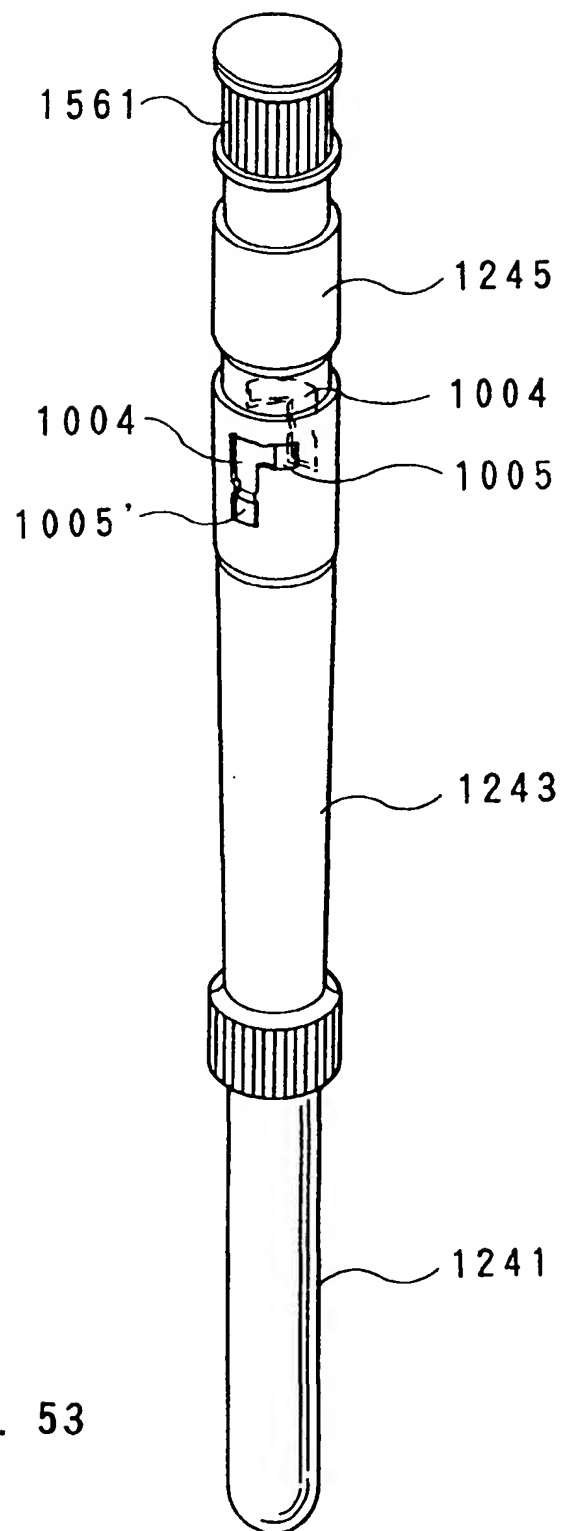
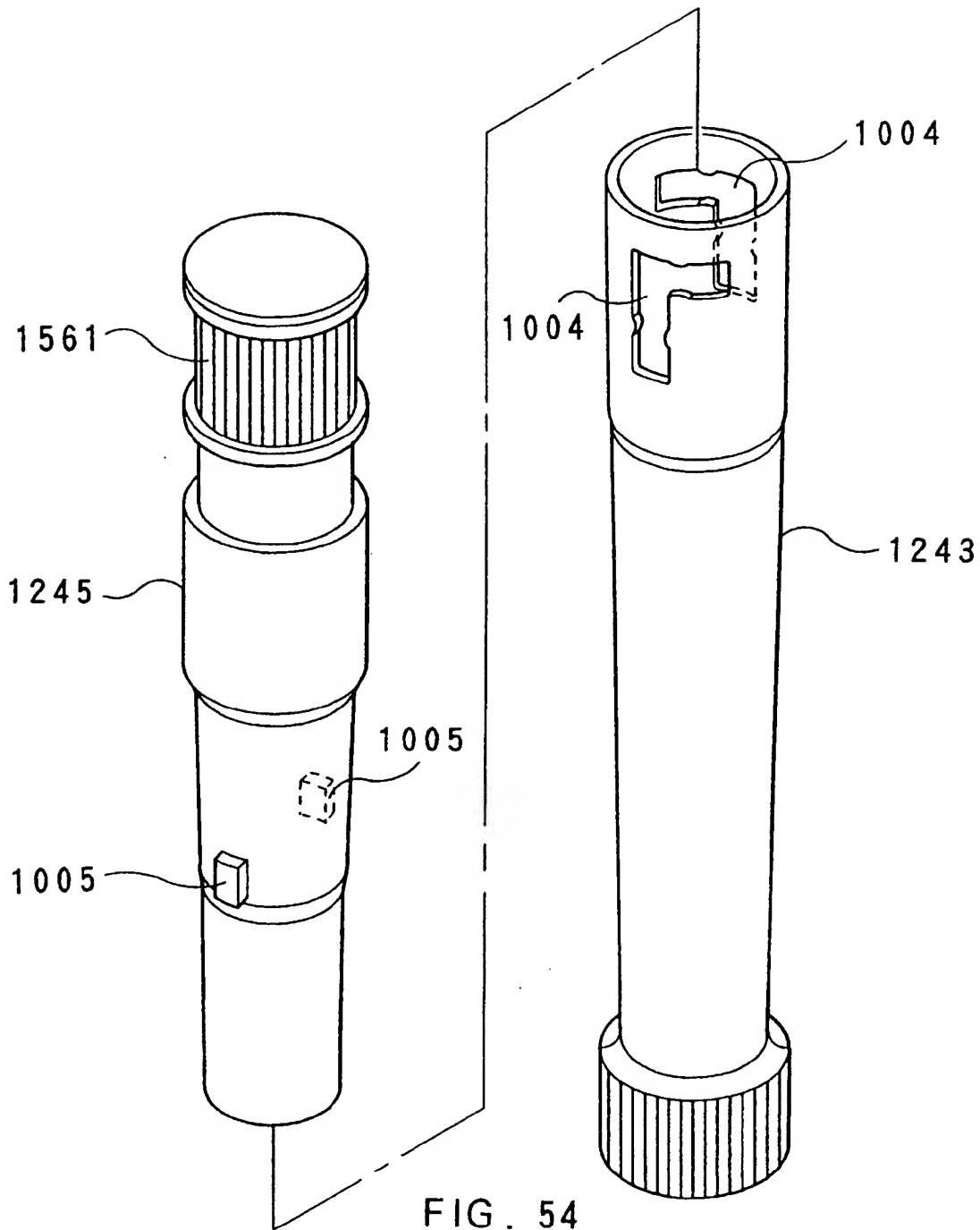


FIG. 53





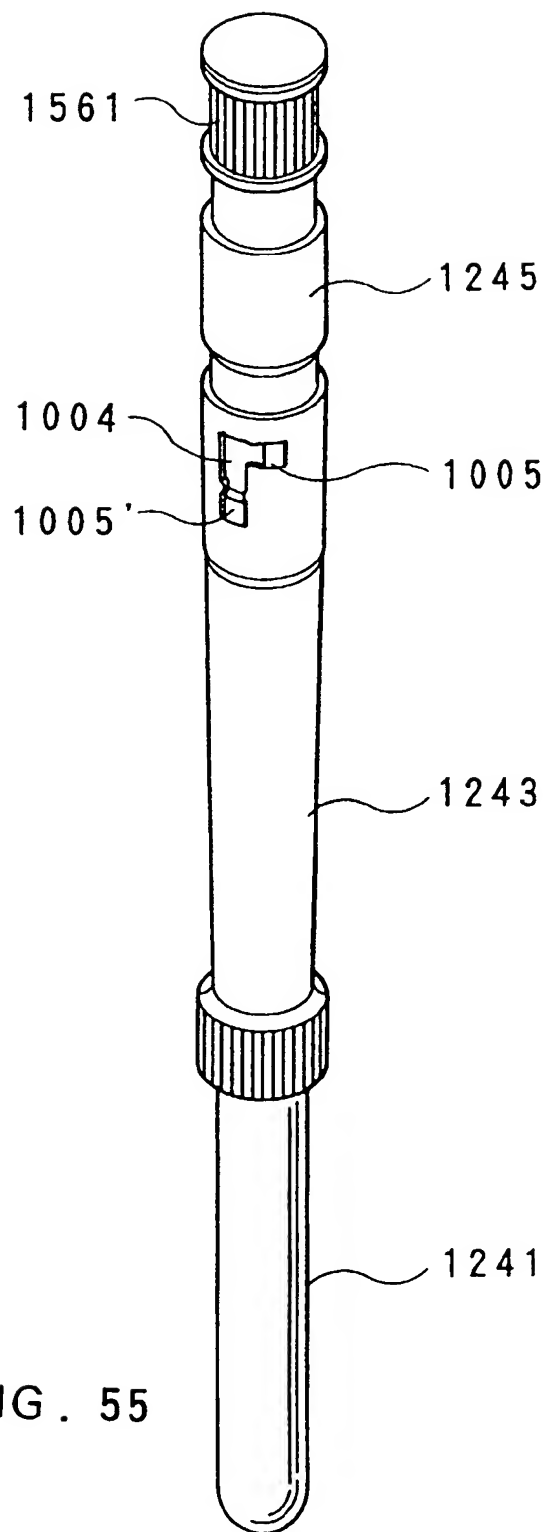
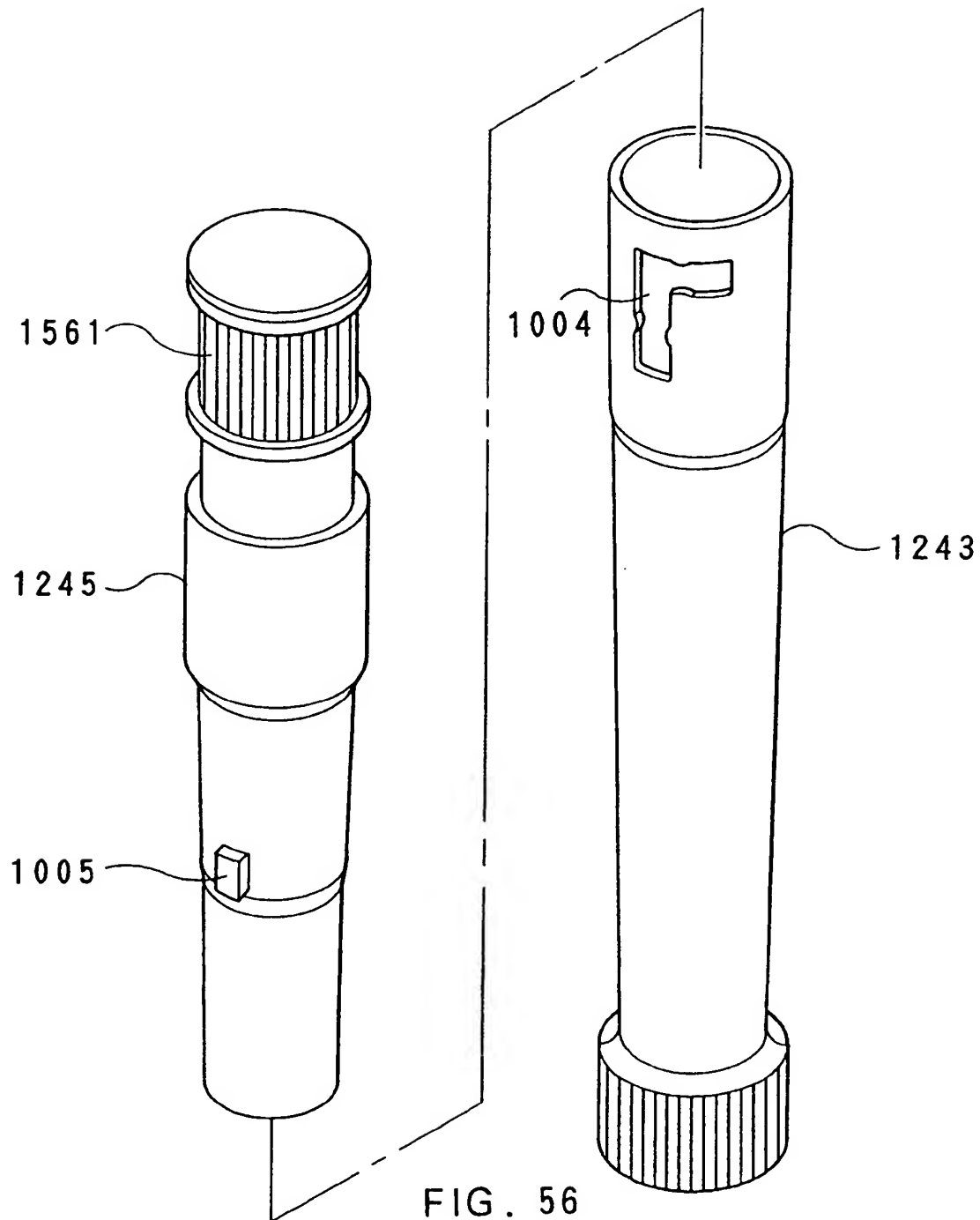


FIG. 55



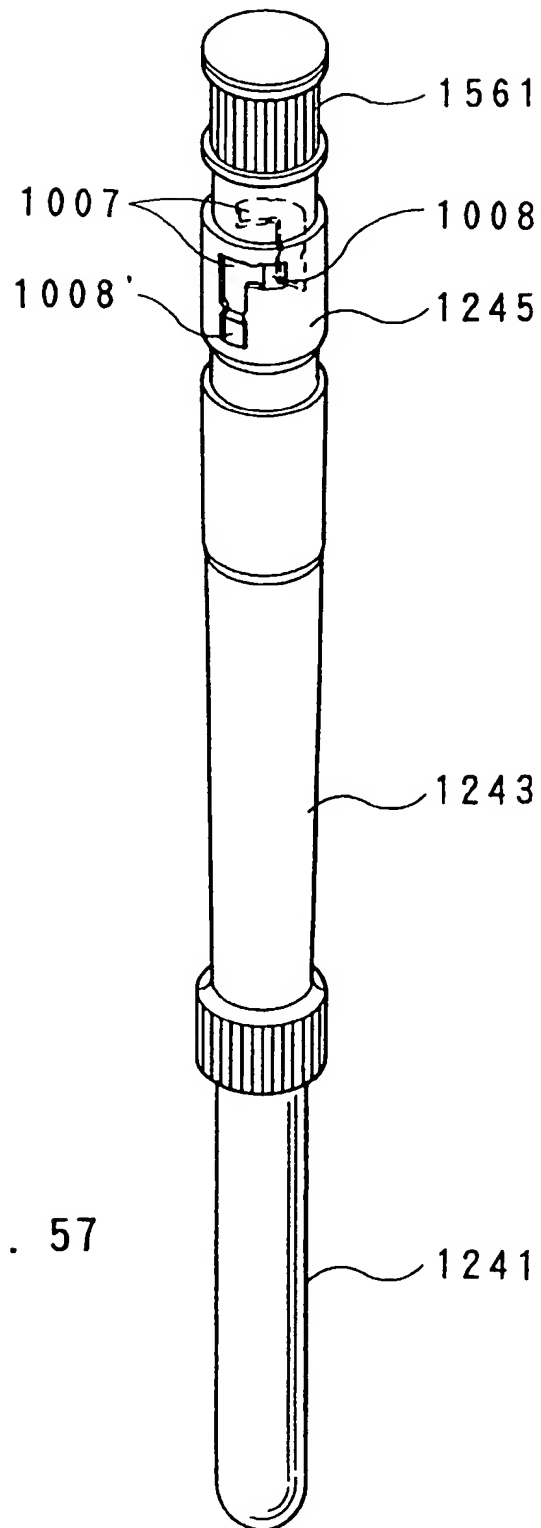


FIG. 57

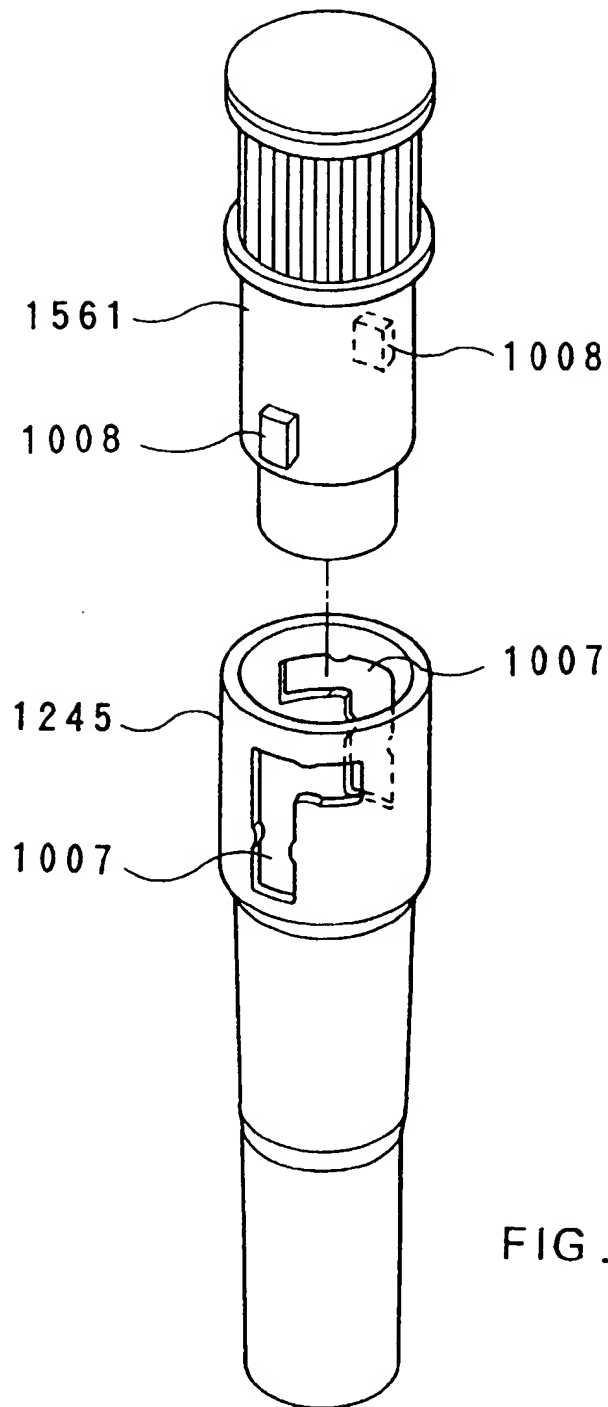


FIG. 58

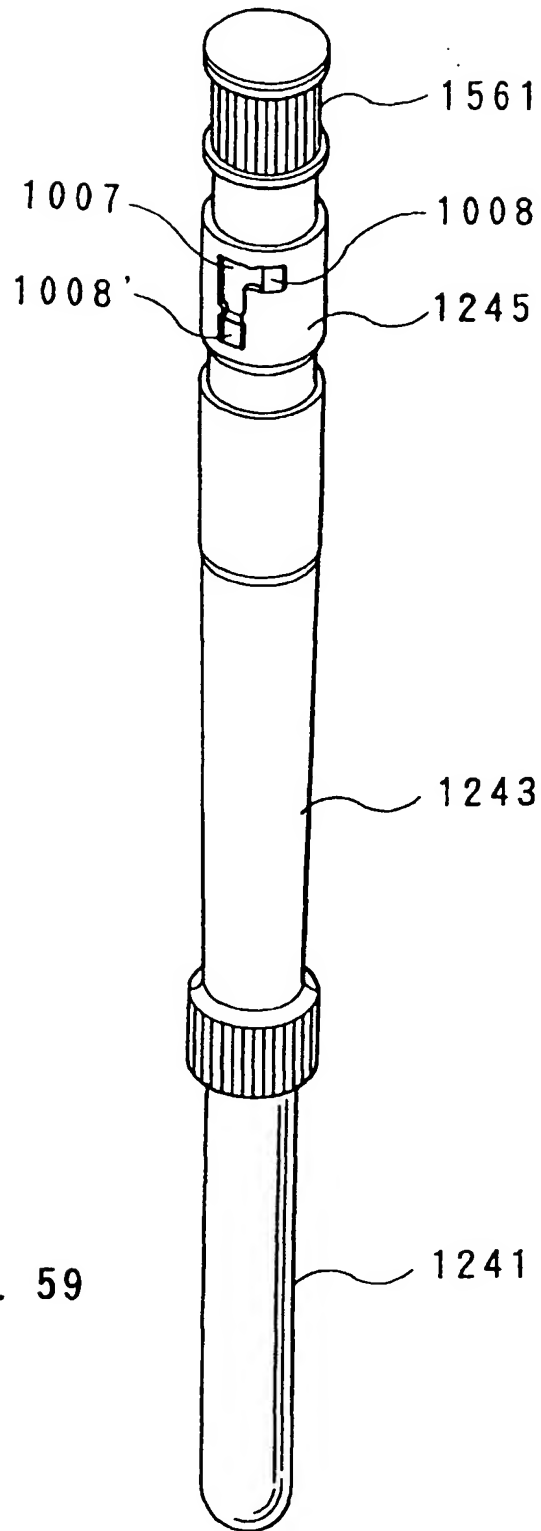


FIG. 59

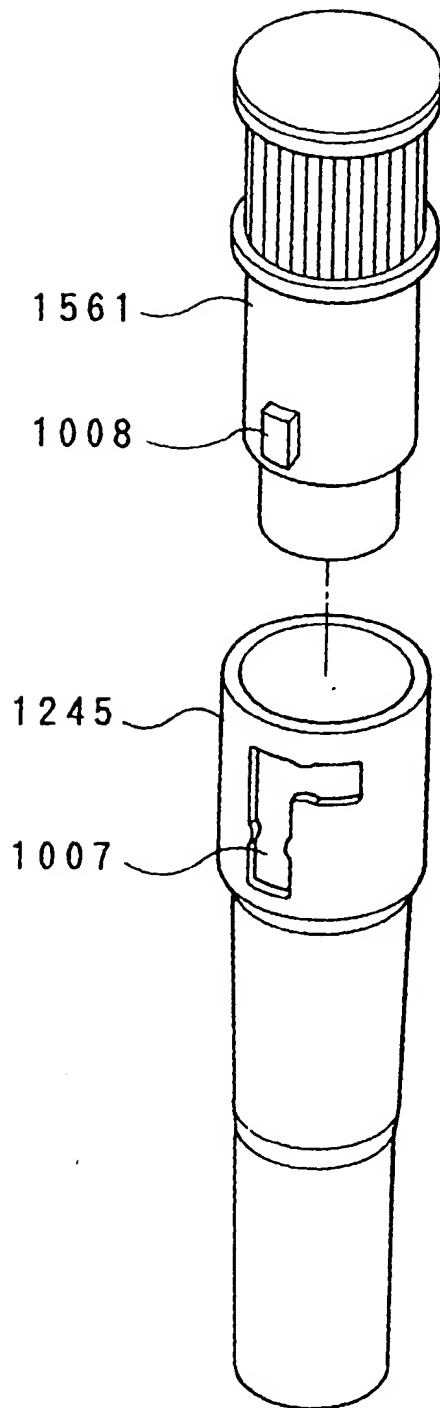


FIG. 60

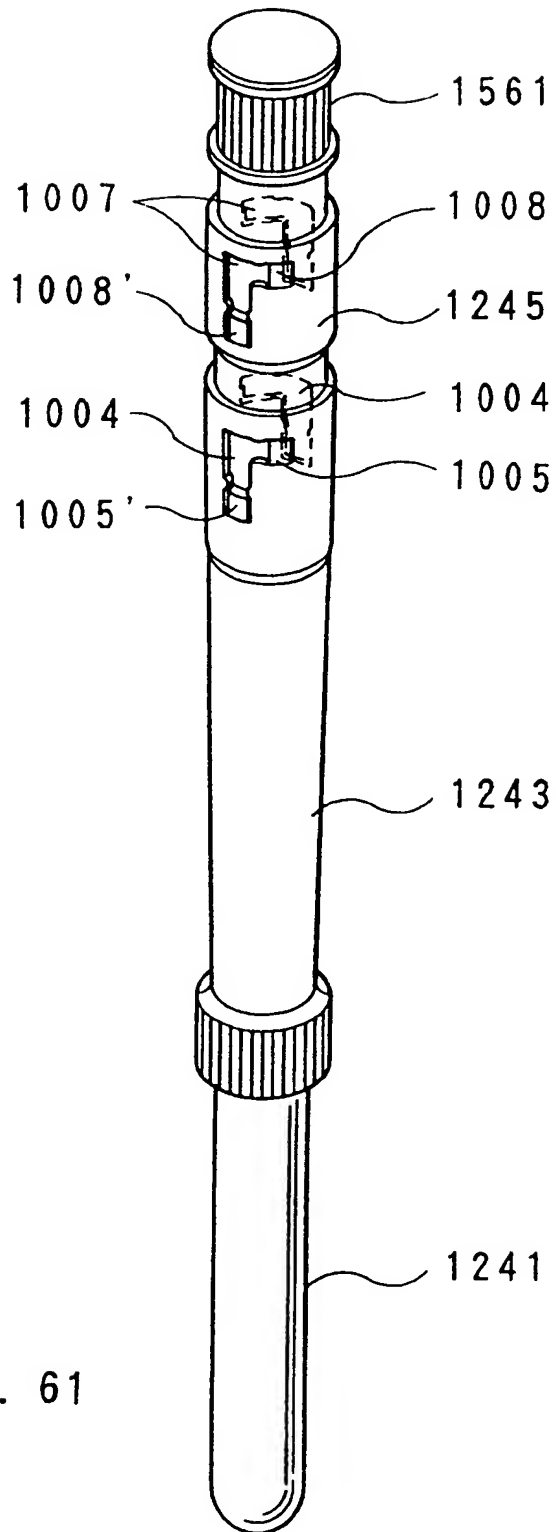
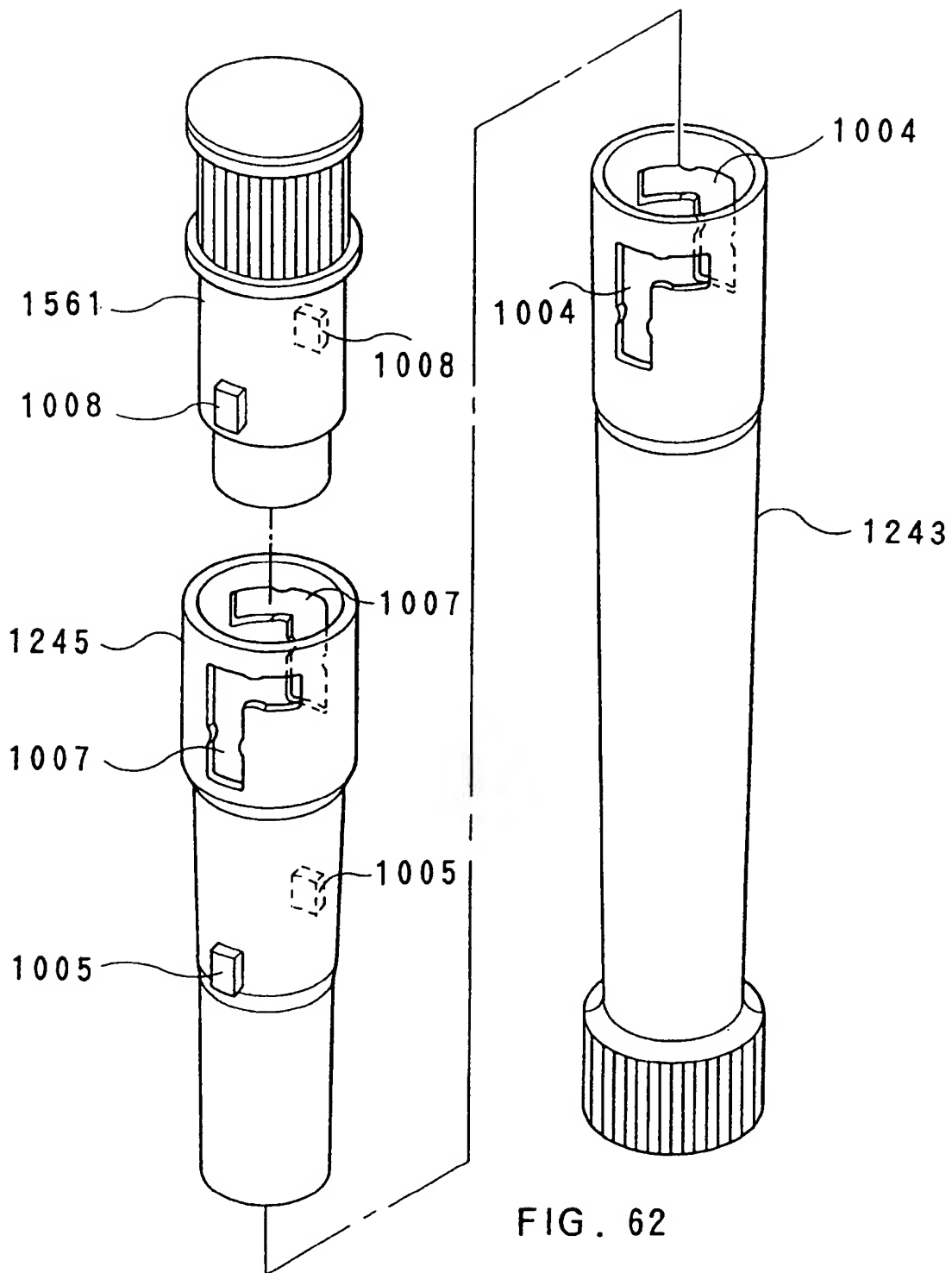


FIG. 61





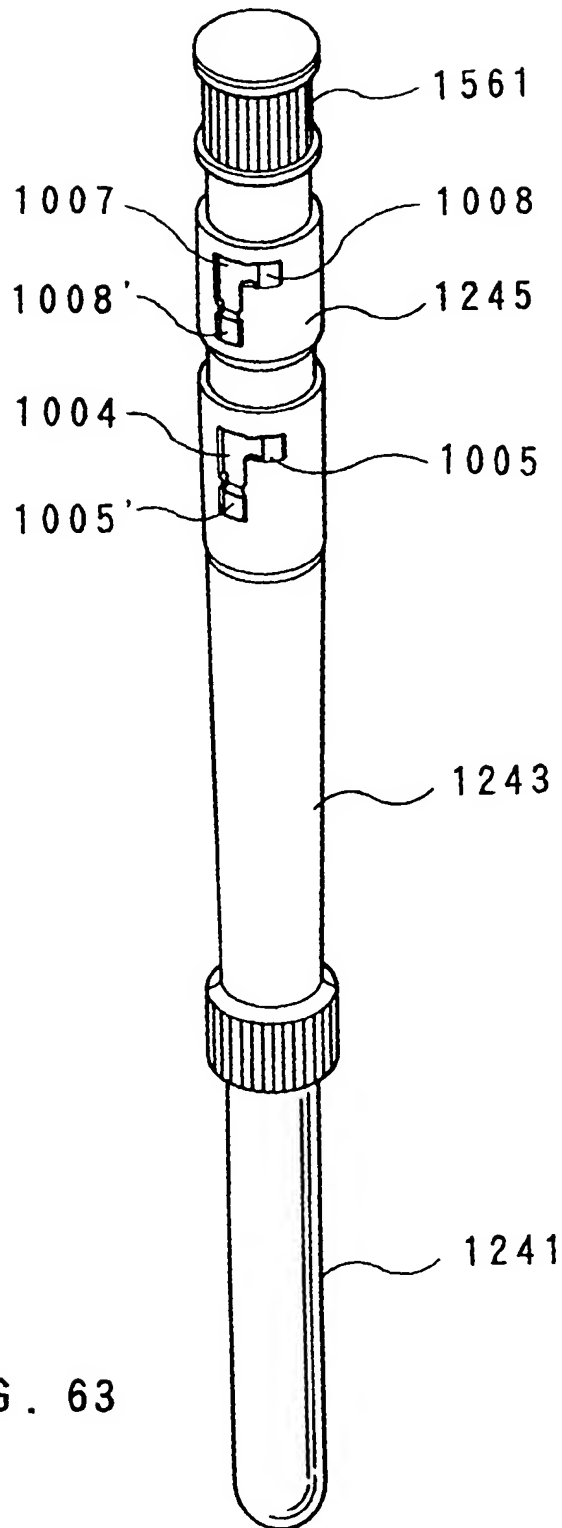


FIG. 63

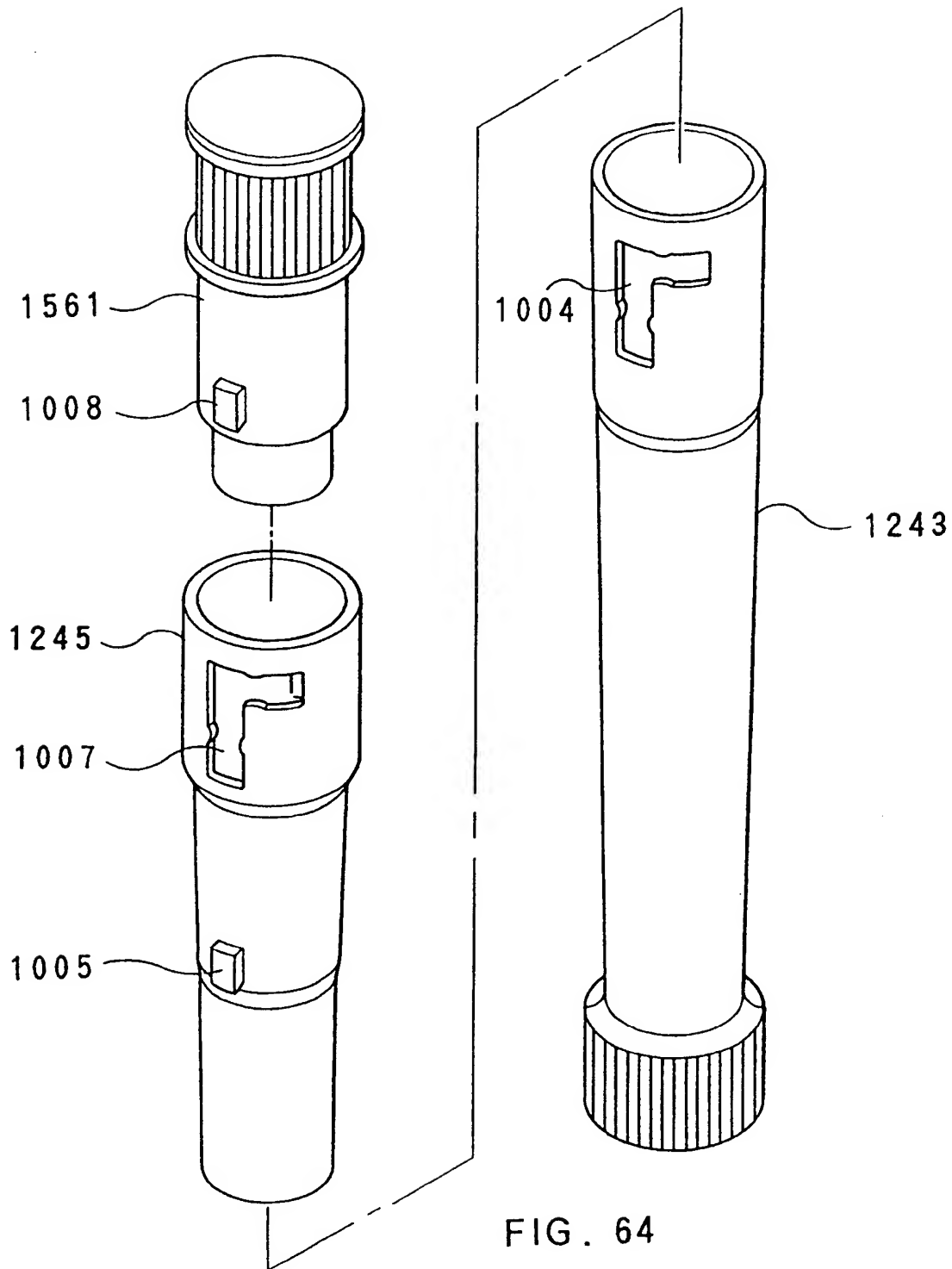


FIG. 65

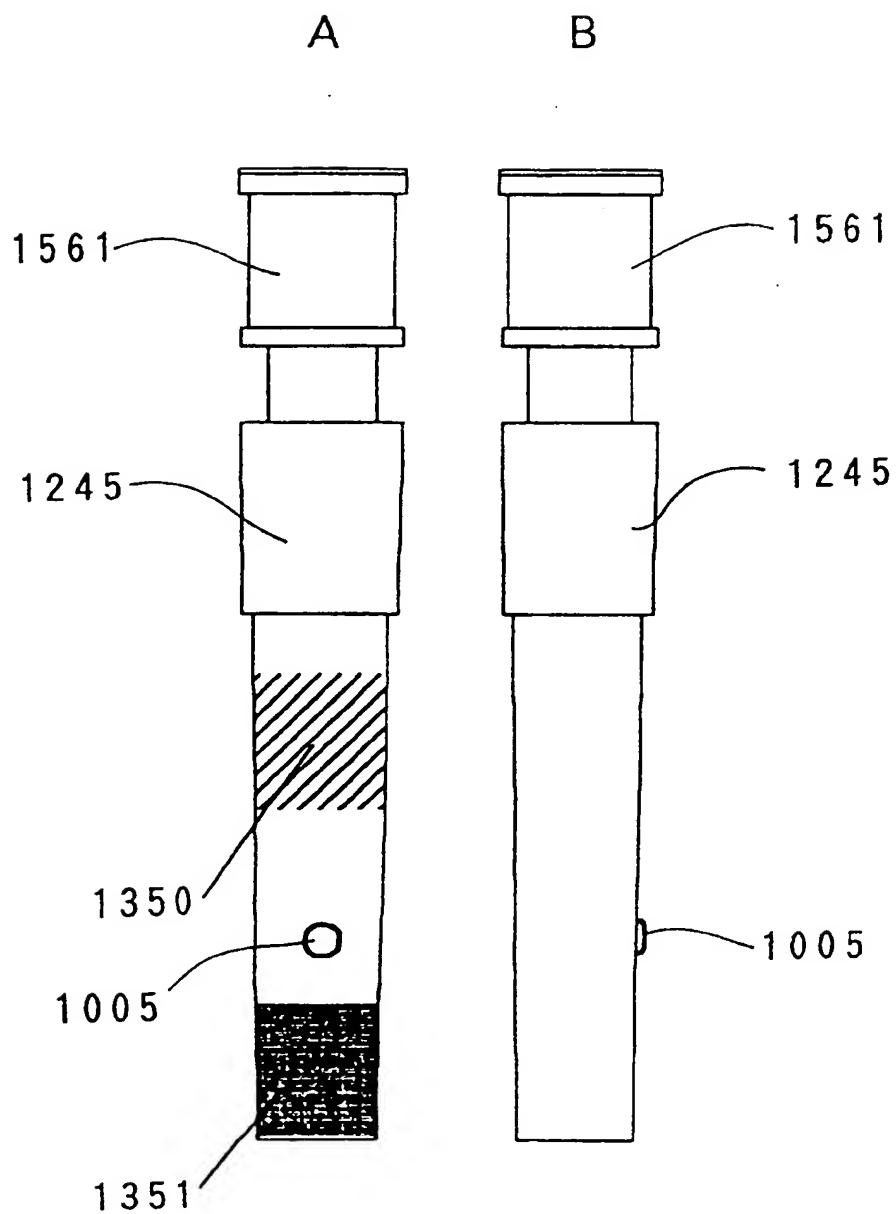


FIG. 66

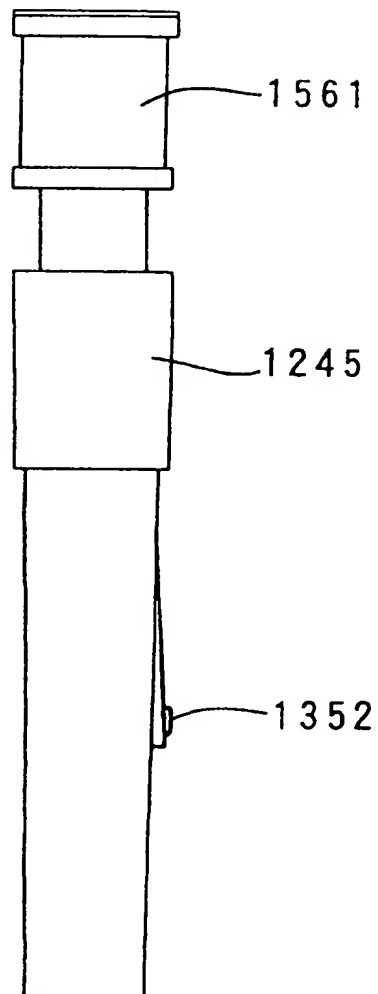


FIG. 67

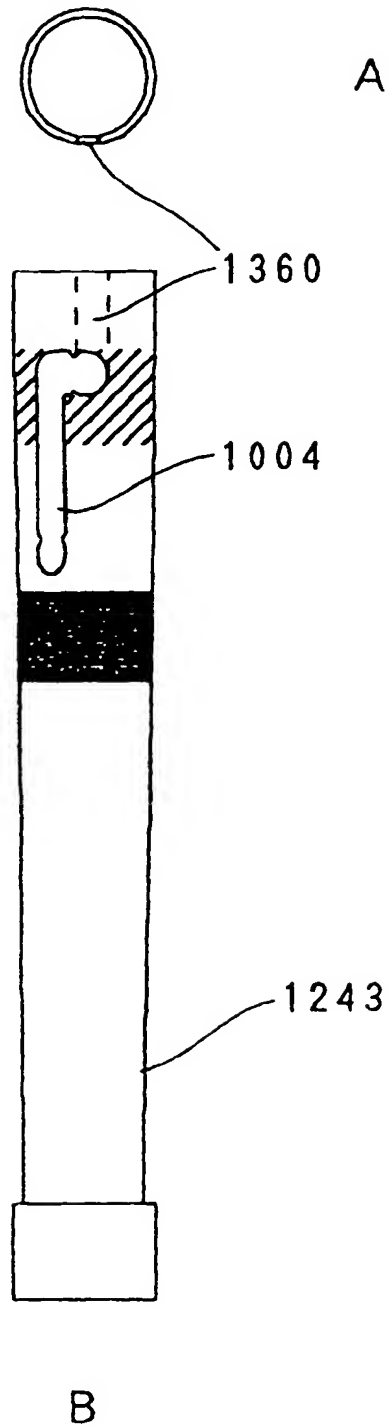


FIG. 68

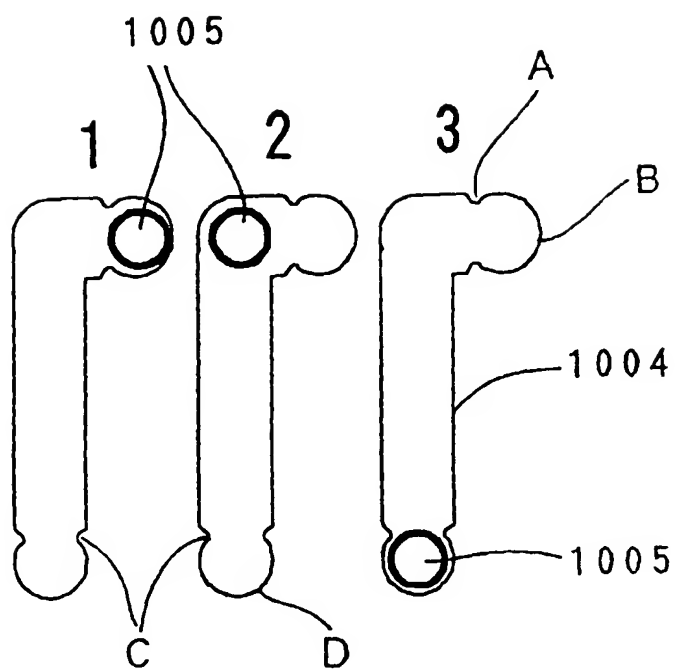
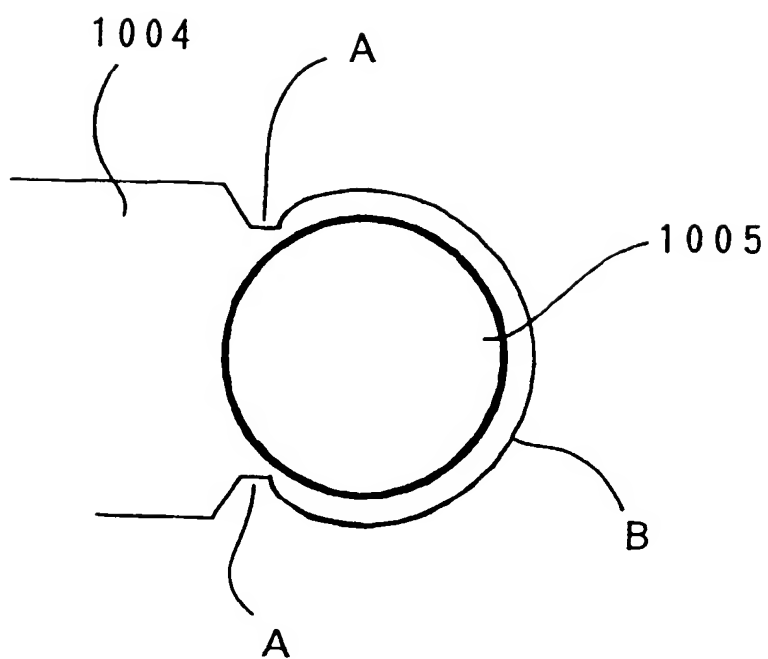


FIG. 69





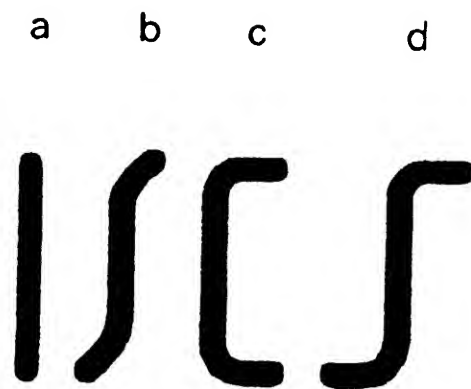


FIG. 70

FIG. 71

RELATION BETWEEN LIVING CELLS AND TURBIDITY

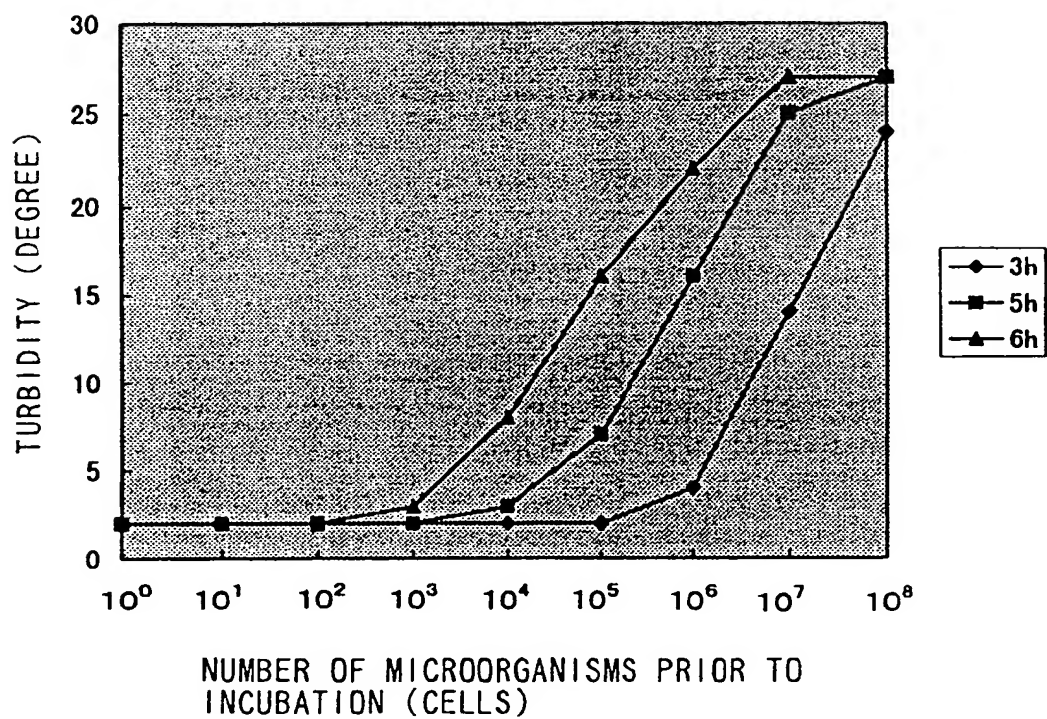
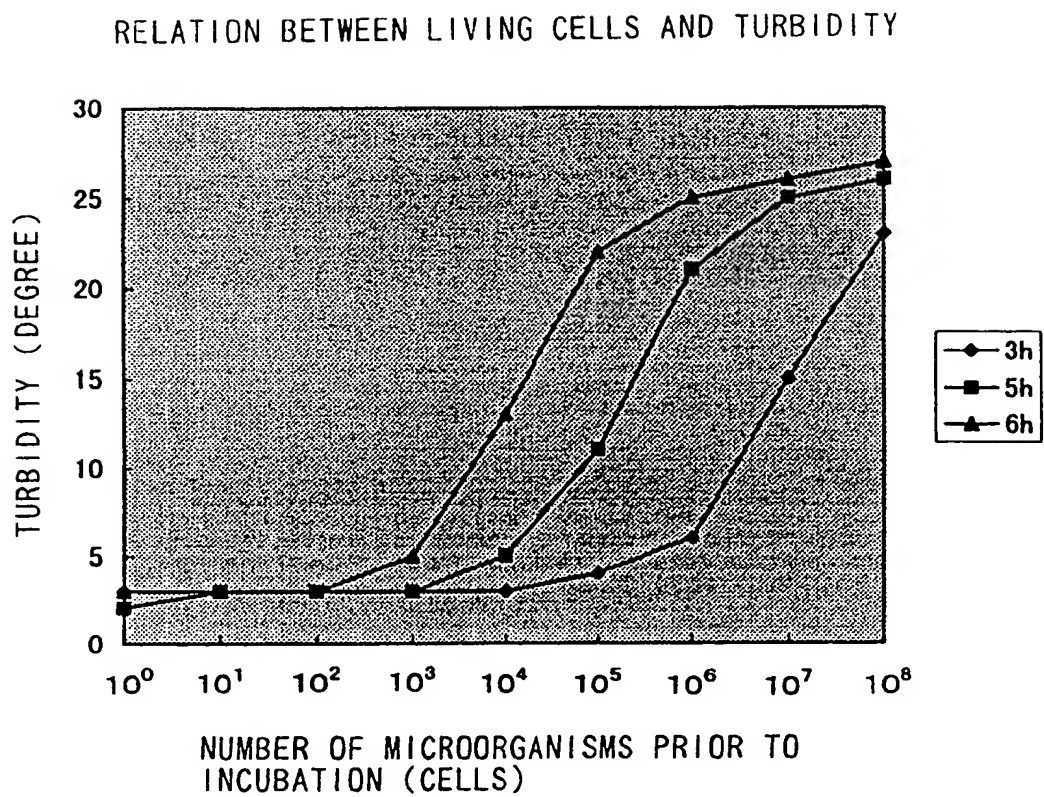


FIG. 72



## INTERNATIONAL SEARCH REPORT

International application No.

PCT/JP97/04066

<b>A. CLASSIFICATION OF SUBJECT MATTER</b> Int.Cl <sup>6</sup> C12M1/28 According to International Patent Classification (IPC) or to both national classification and IPC		
<b>B. FIELDS SEARCHED</b> Minimum documentation searched (classification system followed by classification symbols) Int.Cl <sup>6</sup> C12M1/00-3/10 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)		
<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JP. 7-308184, A (Nihon Millipore Kogyo K.K.), November 28, 1995 (28. 11. 95) (Family: none)	1 - 29
A	JP. 62-171671, A (Kopan S.R.L.), July 28, 1987 (28. 07. 87) & EP. 223745, A & DK. 8605446, A & ES. 2003926, A	1 - 29
A	JP. 62-228147, A (Castle Co.), October 7, 1987 (07. 10. 87) & GB. 2186974, A & US. 4717661, A & US. 4743537, A & GB. 2186974, A & CA. 1290229, A	1 - 29
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Date of the actual completion of the international search February 2, 1998 (02. 02. 98)		Date of mailing of the international search report February 10, 1998 (10. 02. 98)
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